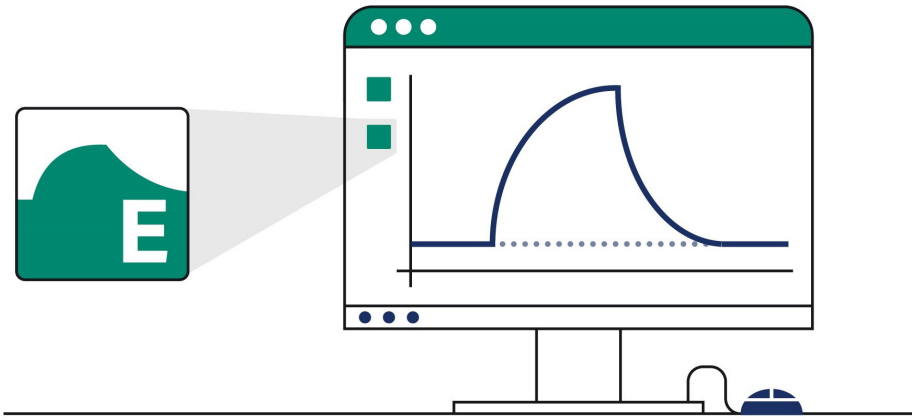
Biacore™ Insight Evaluation Software

User Manual



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1 Introduction

About this chapter

This chapter introduces the Biacore™ Insight Evaluation software.

Biacore Insight Evaluation software is a software package for evaluation of Biacore results stored in a Biacore Insight database. The software is included with Biacore systems that store the results directly in a Biacore Insight database, and can import result files from some other Biacore systems.

In this chapter

1.1 About this manual

Purpose of this manual

The Biacore Insight Evaluation Software User Manual provides a full description of how to use the Biacore Insight Evaluation software for evaluation of results obtained from label-free interaction experiments using Biacore systems.

Note: *Functionality in Biacore Insight Evaluation Software may vary between systems, in particular between 8-channel systems (Biacore 8 series) and 1-channel systems (Biacore 1 series, Biacore T200, Biacore S200).*

Note: *In general, screen illustrations in this User Manual show examples from runs using the Biacore 8 series systems. The detailed appearance may vary for runs from other Biacore systems.*

Software version

The information in this *User Manual* applies to version 6.0 of Biacore Insight Evaluation Software and to most of the extensions available with that version.

For more information about automated data export via an API, included with the ***Data integration*** extension, see *Biacore Insight API Installation and Management Guide 29751155* . See *Biacore Insight GxP User Manual 29312548* for information about the ***GxP*** extension.

Typographical conventions

Software items are identified in the text by ***bold italic*** text.

Hardware items are identified in the text by **bold** text.

Tip: *The text can include clickable hyperlinks to reference information.*

Notes and tips

Note: *A note is used to indicate information that is important for trouble-free and optimal use of the product.*

Tip: *A tip contains useful information that can improve or optimize your procedures.*

1.2 Important user information

Prerequisites

The use of Biacore Insight Evaluation software requires a Microsoft SQL Server database installed according to the instructions in the separate *Biacore Insight Database Installation and Management Guide 29287249*. A valid Cytiva eLicense is also required. Handling of eLicenses is described in the separate *eLicensing Guide 29287250*.

A system administrator familiar with management of Microsoft SQL Server databases is required for database installation and management. Familiarity with SQL Server databases is not required for operation of Biacore Insight Evaluation software.

In order to use Biacore Insight Evaluation software, you must have a general understanding of the use of a personal computer running Windows operating system in the version provided with your product.

1.3 Glossary

Biacore terminology

Terms used in work with Biacore Insight Evaluation software are explained in the following table.

Term	Meaning
Absolute response	The magnitude of the SPR signal measured from the detector baseline.
Adjustment for controls	Adjustment of the sample response for changes in the surface activity during the course of an experiment, by normalizing with respect to control sample responses measured at intervals.
Analysis cycle	A sequence of injections of liquid over the sensor surface. A cycle can be repeated as many times as required during the course of an experiment.
Analyte	The analyte is the interaction partner in solution, that is injected over and interacts with the ligand on the sensor surface. ***Note:*** *The analyte is not necessarily the object of the experimental investigation. For example, an antibody screening experiment may be set up where different antibodies are attached to the sensor surface as ligands, and challenged with antigen injected in solution as analyte. In this case, the object of the investigation is the ligand.*
API	An Application Programming Interface (API) allows different software to communicate and share data with each other. Biacore Insight API enables automated data export from Biacore Insight Evaluation Software.
Association phase	The phase of an analysis cycle where analyte is injected over the sensor surface and (potentially) binds to the ligand.
Baseline	The response level from which sample responses are measured. A baseline is automatically set before each injection in an analysis cycle: baselines may be set at other points in a sensorgram if required.
Binder prediction	Analysis of binding level screen using Biacore Intelligent Analysis™.

Term	Meaning
Blank subtraction	Subtraction of the response from a blank sample (usually buffer) from that from a test sample, to eliminate components of the response that are common to both samples.
Capture	Attachment of ligand to the sensor surface by high affinity binding to an immobilized capturing molecule. Attachment by capture is normally reversible.
Capturing molecule	A molecule that is permanently attached to the sensor surface with the purpose of capturing ligand by high affinity binding.
Channel	A single path for liquid flow over the sensor surface.
Data set	The results of one or more runs, opened for evaluation in one evaluation session.
Dissociation phase	The phase of an analysis cycle immediately following the association phase, when buffer flows over the sensor surface and any bound analyte may dissociate spontaneously.
EC_{50}	Half-maximal effective concentration, a measure of the potency of a drug.
Enhancement molecule	A secondary analyte injected after the main analyte, intended to enhance the response and/or specificity of the first analyte binding.
Flow cell	The region of a channel where detection occurs. Different systems have different flow cell arrangements and options.
Immobilization	Permanent attachment of ligand or capturing molecule to the sensor surface, normally by covalent coupling.
Ligand	The ligand is the interaction partner attached to the surface. Attachment may be through covalent coupling (***immobilization***) or high affinity binding to an immobilized capturing molecule (***capture***). ***Note:*** *Use of the term **ligand** in Biacore contexts does not imply that the molecule is a ligand for a cellular receptor.*
Parallel flow	A flow pattern where liquid flows through multiple channels in the same way and at the same time.
PLA	Parallel Line Analysis (also called Parallel Line Assay), a method for determining the potency of a drug relative to a reference standard.
Quality prediction	Analysis of affinity screen using Biacore Intelligent Analysis.

Term	Meaning
Reference subtraction	Subtraction of the response from the reference surface from that from the active surface, to eliminate components of the response that are common to both surfaces.
Reference surface	The sensor surface in the flow cell used as a reference.
Regeneration	The act of removing all non-covalently attached material from the sensor surface (usually by injection of a regeneration solution) in preparation for the next analysis cycle.
Relative response	The magnitude of the SPR signal relative to a chosen reference point (usually the baseline before sample injection).
Resonance unit (RU)	The unit of measurement for the SPR response. As a rough approximation, 1 RU is equivalent to a change in protein concentration of 1 pg/mm^2 on the surface of Sensor Chip CM5. This equivalence varies with different analytes and different sensor chip types.
Report point	A point measurement of the response at a specified time on the sensorgram. The response is calculated as the median response over a short window (typically 5 s), centered on the specified time point. ***Note:*** *Median response values are used in Biacore Insight Evaluation software. Average values are used for report points in some other Biacore systems.*
Sensor chip	A gold-covered glass slide to which one of the interactants (the ligand) is attached.
Sensor surface	The surface of the sensor chip on which the interaction being studied takes place.
Sensorgram	A graph of response against time during one analysis cycle.
Serial flow	A flow pattern where the same liquid flows through two or more flow cells, one after another.
SPR	Surface plasmon resonance, the detection principle used in Biacore instruments.

1.4 Scope of Biacore Insight Evaluation software

Intended use

Biacore Insight Evaluation software provides tools for evaluating results of label-free interaction analysis experiments, stored in a Biacore Insight database. Runs from systems that store the results in a Biacore Insight database may be evaluated directly. Results stored in ***.blr*** files must first be imported to the database.

Biacore Insight Evaluation software is the only option provided by Cytiva for evaluation of results from systems such as Biacore 1 series and Biacore 8 series systems, which store the results directly in the Biacore Insight database. Other Biacore systems are supplied with a system-specific evaluation software, and Biacore Insight Evaluation software provides an alternative environment for evaluation of these results. The functionality offered by Biacore Insight Evaluation software and the system-specific evaluation software may not be fully equivalent, and Biacore Insight Evaluation software is most useful for users who want to evaluate results from different systems in a common environment.

NOTICE

Even when nominally equivalent functions are provided by Biacore Insight Evaluation software and evaluation software from other Biacore systems, the exact results of the evaluation can differ. This is due to differences in the way the data is handled, for example:

- Sensorgram display in Biacore Insight Evaluation software uses a smoothing algorithm that can eliminate spikes in the sensorgram.
- Report points included in result files (***.blr***) are ignored when the files are imported to the Biacore Insight database. Report points set in Biacore Insight Evaluation software may not be equivalent to those in the ***.blr*** file.
- Report points are calculated as the median value in the report point window in Biacore Insight Evaluation software, but as the average value in most systems that store results in ***.blr*** files.

Exercise caution in comparing results between Biacore Insight Evaluation software and system-specific evaluation software.

Software extensions

Biacore Insight Evaluation software is provided as a basic package with optional extensions for specialized functionality. The basic software and extensions are licensed separately. In order to access the functionality in a specific extension, the following requirements must be met:

- There must be an eLicense available for the extension.

- The extension must be activated in the software when the user logs in (see *Section 2.1 Starting the Biacore Insight Evaluation software, on page 21*).

Currently available extensions are summarized in the table below. The version number refers to the Biacore Insight Evaluation software version.

Extension	Functionality	Available from version
Biacore Intelligent Analysis	Support for evaluation of binding level screens and affinity screens using prediction models. All functionalities that are part of the ***Extended Screening*** extension are also included.	4.0
Concentration & Potency	Support for concentration measurements using calibration curves. Support for PLA (Parallel Line Analysis) and EC_{50} (half-maximal effective concentration) determination.	2.0
Data Integration	Enables use of Biacore Insight API server for automated data integration, and allows manual export of evaluations in JSON or XML format from Biacore Insight Evaluation Software. Instructions for installing, configuring and integrating with the Biacore Insight API for automated data export are provided in the *Biacore Insight API Installation and Management Guide (29751155)*.	3.0 (manual export) 6.0 (automated export)
Epitope Binning	Support for setup and evaluation of epitope binning experiments.	3.0
Extended Screening	Predefined methods for evaluation of fragment screening runs. Functionality optimized for screening of low molecular weight compounds.	1.0

Extension	Functionality	Available from version
GxP [1]	Provides support for work in regulated environments, including restricted access for routine users, and audit trails. Functions in the ***GxP*** extension are described in the separate *Biacore Insight GxP User Manual 29312548*.	2.0

[1] The GxP extension is not available to users who do not have a GxP role assigned to them by the database administrator. See the *Biacore Insight Database Installation and Management Guide 29287249* for more information.

Functions that are restricted to an extension are stated as such.

Support for different Biacore systems

The current version of Biacore Insight Evaluation software can be used to evaluate data from the following Biacore systems:

System	Type	Version	Support[1]
Biacore 8 series: Biacore 8K Biacore 8K+	8-channel	1.0 or later	Full functionality
Biacore 1 series: Biacore 1K Biacore 1K+ Biacore 1S+	1-channel	5.0 or later	Full functionality
Biacore T200	1-channel	1.0 or later	Limited functionality (see below)
Biacore S200	1-channel	1.0 or later	Limited functionality (see below)

[1] Support refers to evaluation of result files from interaction analysis experiments. Results from ligand immobilization runs and from maintenance and service tools are handled in the Control Software for the respective systems.

Result files (`.blr`) from Biacore T200 and Biacore S200 systems must be imported into the Biacore Insight database in order to be evaluated. Evaluation files (`.ble`) cannot be imported.

The following limitations and adaptations apply to evaluation of result files from Biacore T200 and Biacore S200 systems in Biacore Insight software:

- Import is not possible for all files. A message describing the reason is shown in such cases.

- Some applications supported in the Evaluation Software for Biacore T200 and Biacore S200 systems are not supported in Biacore Insight software.
- Injections of type ***InjectAndRecover*** are treated as ***Inject and elute*** injections.
- Injections of type ***Merged injection*** are supported but are treated as simple ***Analyte*** injections.
- Injections of type ***Sample*** are treated as ***Analyte*** injections.
- Biacore Insight software treats all samples in assay steps with purpose ***Control*** as positive controls. The assay step purpose ***Control*** is changed to ***Analysis*** and the samples are set to positive controls. The control status for samples can be edited in the ***Variables*** table, which automatically appears upon evaluation of result files from Biacore T200 and Biacore S200 systems containing controls.
- Keywords in the imported file that are not available as predefined variables in Biacore Insight evaluation are imported as user-defined variables.

Note: *Screenshots in this manual show evaluation of runs from a Biacore 8 series system, unless otherwise stated. Detailed appearance may vary for data from other Biacore systems.*

1.5 Data storage and management

Introduction

All data handled by Biacore Insight Evaluation software is stored in a local or network Microsoft SQL Server database, referred to as Biacore Insight Evaluation Database. Instructions for installing and managing the database are given in the separate *Biacore Insight Evaluation Database Installation and Management Guide*.

Data stored on a network is accessible to all users of the Biacore Insight Evaluation software subject to restrictions imposed by the Biacore Insight GxP functionality. Data stored locally may be accessible from other computers depending on local IT policies.

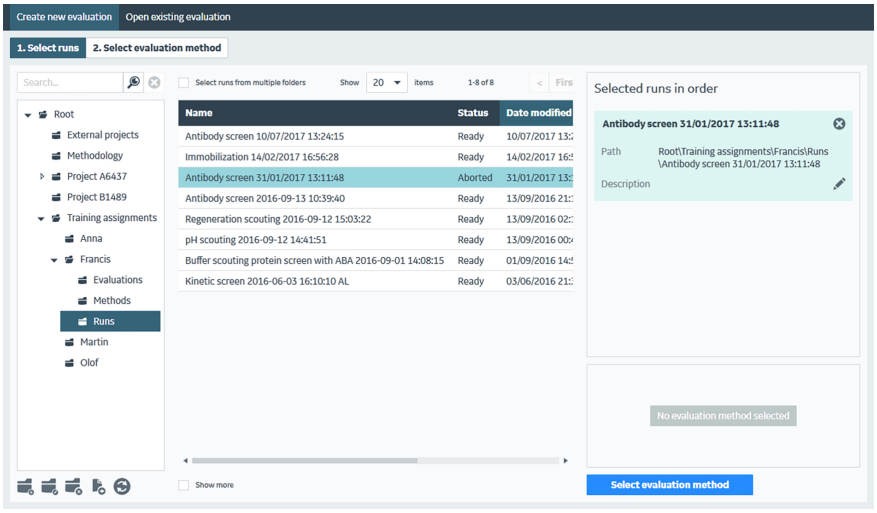
Results from Biacore 1 series and Biacore 8 series systems are stored directly in the database. Results from other Biacore systems must be imported to the database before they can be evaluated with Biacore Insight Evaluation software.

Note: *If multiple users work on the same data at the same time, the user who first saves changes can save the item with the original name. Other users must save their changes as a new item, using* ***Save as****.*

Data organization

Data is organized in folders in the database. All runs, evaluations, and user-defined evaluation methods (collectively referred to as *database objects*) are stored in user folders. Storing user-defined objects directly under the root folder is not recommended.

The folder structure is displayed in the left-hand panel in appropriate workspaces, with the contents of the selected folder in the main panel. Details of selected items are shown to the right.



Select ***Show more*** in the contents panel to show additional details.

Note: *All folders and objects in the database are accessible to all users unless restrictions are implemented through IT policies or GxP restrictions.*

Time stamps

Time stamps are created as local time on the computer where the stamped operation is performed, and displayed with an offset to local time in a different time zone (for example, a run performed at 11:00 AM EST will be shown as 8:00 AM PST).

Managing database objects

Manage objects in the database according to the instructions in the following table.

Operation	Instruction
Move object	Drag the object from the list in the main panel of the workspace to a different folder in the left-hand panel.
Rename object	Click twice on the object name in the main panel of the workspace to highlight the name and enter a new name. ***Note:*** *Click twice with a longer interval than double-clicking.*
Remove object	1. Scroll to the right if necessary. 2. Choose ⊗ ***Remove*** in the ***Action*** column. ***Note:*** *The **Remove** button may not be visible depending on the database security settings. See the Biacore Insight Database Installation and Management Guide (29287249) for details.*
Export a single object	1. Scroll to the right if necessary. 2. Select ➔ ***Export*** in the ***Action*** column. The file is exported in a proprietary format intended for import to another Biacore Insight database.

Operation	Instruction
Export multiple objects	1. Select a folder or perform a search to define the objects to be exported. All objects shown on the current page will be included in the export. 2. Choose ***Show more*** in the right-hand panel of the workspace. 3. Click ***Export objects***, and select whether you want all objects in the same folder, or if you want the folder structure to be preserved. The files are exported in a proprietary format intended for import to another Biacore Insight database. ***Tip:*** *Use the search function to export objects of different types (runs, run methods, evaluations, evaluation methods) in a single operation.*
Import object (s)	1. Navigate to the destination folder in the database. 2. Select  ***Import*** from the icons at the bottom of the panel. 3. Select file(s) to import. Result files from Biacore T200 and Biacore S200 and files exported from a Biacore Insight database can be imported.

Managing folders

To manage folders, right-click on the folder in the left-hand panel or use the icons at the bottom of the panel.

Operation	Icon	Comments
Add folder		Enter the name for the folder.
Rename folder		Enter the new name for the folder.
Remove folder		You cannot remove a folder that contains subfolders or other objects.

Operation	Icon	Comments
Import		Use this function to import result files from Biacore T200 and Biacore S200 and files exported from a Biacore Insight database to the current folder.
Refresh		Refreshes the display.

Searching for database objects

To search for objects in the database, enter a search term in the ***Search*** field. Search terms are not case-sensitive. Enclose phrases containing spaces in single or double quotation marks to search for the entire phrase (for example, searching for ***new evaluation*** will find both ***new*** and ***evaluation***, but searching for ***"new evaluation"*** will find only the complete phrase ***new evaluation***). Choose the appropriate search criteria, then click ***Search***. The available criteria may vary according to the type of object selected. You can restrict the search according to multiple criteria:

Type	Parameter	Description
Search for	***Runs***	Finds runs with the search term in the name. If you are in the ***Create new evaluation, 1. Select runs*** workspace, you can select runs from the search result.
	Run methods	Finds immobilization and analysis methods with the search term in the name. ***Note:*** *Immobilization and analysis methods cannot be opened in Biacore Insight Evaluation software.*
	Evaluations	Finds evaluations with the search term in the name. If you are in the ***Open existing evaluation*** workspace, you can open an evaluation from the search result.
	Evaluation methods	Finds evaluation methods with the search term in the name. If you are in the ***Create new evaluation, 2. Select evaluation method*** workspace, you can select an evaluation method from the search result.

Type	Parameter	Description
Search within name and	***Description***	Finds objects where the object name or the description contains the search term.
	Ligand	Finds objects where the object name or the name of the immobilized molecule contains the search term. ***Note:*** *For runs that use captured ligand, searching for* ***Ligand*** *will find the capturing molecule.*
	Solution	Finds objects where the object name or the name of the ***Solution*** parameter in any command (including ***Capture*** commands) contains the search term.
	Regulated procedure	Finds objects where the object name contains the search term, or the object is derived from a regulated procedure with a name that contains the search term. This option is only available when the ***GxP*** extension is active.
Filter on	***Date***	Finds objects modified in the specified date range.
	Users	Finds objects modified by any of the specified user(s).
	Instrument id	Finds results and evaluations containing data from the selected instrument(s). Select the instrument id(s) from the list that appears when this option is checked. The list includes all instruments that have been used for runs in the current database. ***Note:*** *Evaluations may contain data from more than one instrument.*
	Selected folder and subfolders only	Searches only the selected folder and subfolders. If this option is not selected, the search is performed on the whole database.

2 Overview of Biacore Insight Evaluation software

About this chapter

This chapter provides a general description of the Biacore Insight Evaluation software. Details of specific evaluation tasks are given in following chapters.

In this chapter

2.1 Starting the Biacore Insight Evaluation software

Introduction

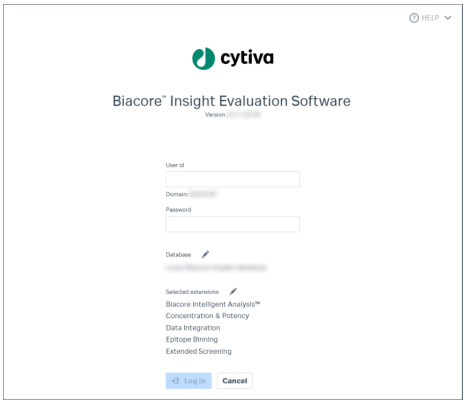
The Biacore Insight Evaluation software may be run on any computer that is connected to an appropriate database, provided that a valid license is obtained. The software does not require connection to a Biacore instrument.

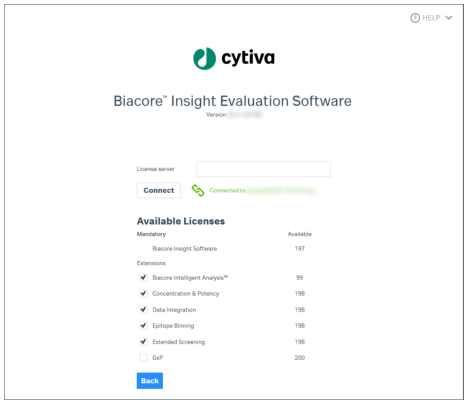
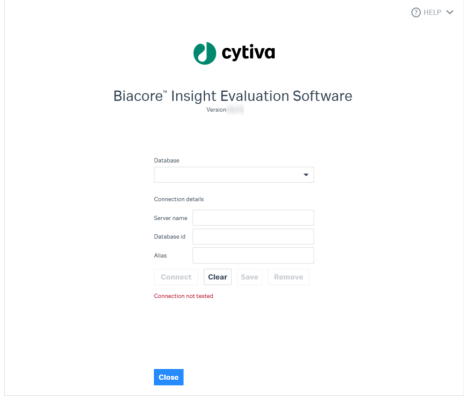
One instance of the Biacore Insight Evaluation software can process one data set (derived from one or several runs). To evaluate multiple data sets at the same time, start multiple instances of the software.

Procedure

Follow the steps below to start the Biacore Insight Evaluation software:

Step	Action
1	Start the Biacore Insight Evaluation software. *Result:* The login dialog is displayed.



Step	Action
2	Make sure that the correct license server, database, and software extensions are selected.[1] A warning symbol next to the ***Selected extensions*** list indicates that the connection to the license server has not been specified or has been lost. Click the pen icon next to ***Selected extensions*** to specify the server details and select extensions.  Enter the license server name and click ***Connect***. Contact your system administrator if you need assistance.
3	Select the software extensions you wish to use.
4	Click ***Close*** when you have entered the details.
5	Click the pen icon next to ***Database*** to change the selected database. Contact your system administrator if you need assistance.[1] 

Step	Action
	Note: *If you create a connection to a database that has not previously been accessed from this computer, click* ***Connect*** *then* ***Save*** *to establish and save the connection before closing this dialog.*
6	Enter your Windows account credentials as ***User id*** and ***Password***. ***Note:*** *Biacore Insight Evaluation software does not support Windows Fast User Switching.*
7	Click ***Log in***.

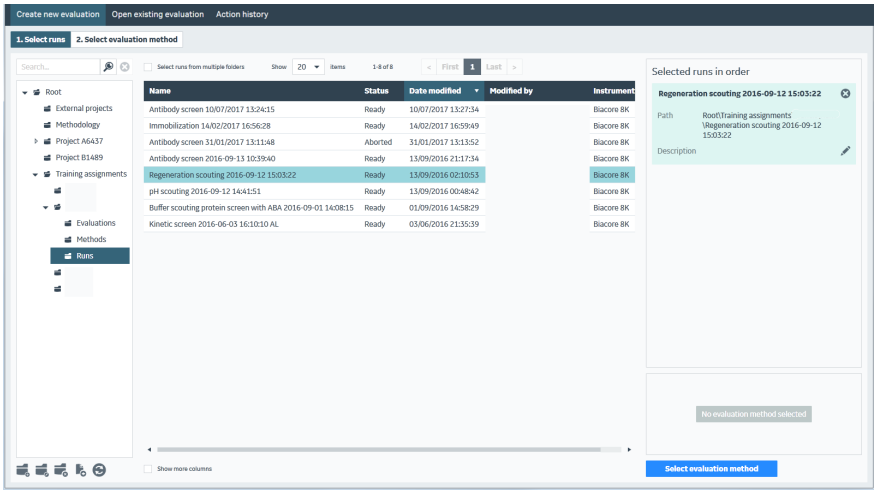
2.2 Opening runs for evaluation

Introduction

This section describes how to open runs for evaluation.

Runs are opened for evaluation together with an evaluation method, which defines procedures that will be applied automatically as far as possible (see *Chapter 11 Evaluation methods, on page 280* for details). This allows evaluation to be standardized for runs of the same type by applying the same evaluation method.

Multiple runs from the same type of Biacore system can be evaluated together as a single data set. Runs from different system types cannot be evaluated together.



Creating new evaluations

Follow the steps below to create a new evaluation.

Note: *Creating a new evaluation will close any current evaluation. Start additional instances of the software if you want to perform several evaluations at the same time.*

Step	Action
1	Select ***Create new evaluation*** at the top of the workspace and go to ***Select runs***.
2	Navigate to the required folder and choose the run(s) to be evaluated. Use **Shift-click** or **Ctrl-click** to select adjacent or non-adjacent runs respectively. *Result:* The runs are added to the ***Selected runs in order*** pane.

Step	Action
	Note: *If multiple runs are selected, the runs will be opened in the order listed in the* ***Selected runs in order*** *pane.*
3	To open multiple runs from different folders in the same session, select ***Select runs from multiple folders***. If this option is not selected, the ***Selected runs in order*** list will be cleared whenever you navigate away from the current folder.
4	Choose ***Select evaluation method*** (alternatively double-click on the run in the previous step). *Result:* The workspace lists evaluation methods.
5	Choose either ***User defined*** or ***Predefined***. User defined methods are custom evaluation methods created by users, and may be stored in any folder in the database. Predefined methods are stored in a reserved location outside the database, and do not appear in any database folder. Select the method you want to use.
6	Click ***Open*** (alternatively double-click on the method in the previous step). *Result:* The data set is opened and the selected evaluation method is applied to the data.

For evaluation of multiple runs, the order in which files are opened has no effect on the evaluation results except for the run identification numbers. You can use the arrow icons in the ***Selected runs in order*** pane to adjust the order if desired, for example, to maintain consistent run numbering between different sessions.

Note: *You can only select a single evaluation method. The method will be applied to the whole data set opened in the session.*

You cannot add runs to an already open evaluation session.

Opening existing evaluations

To open saved evaluations, choose ***Open existing evaluation*** at the top of the workspace and navigate to the required evaluation. Evaluation methods cannot be applied to existing evaluations.

When the ***GxP*** extension is in use, click the action button ***View version history*** to view the version history for the evaluation.

Note: *Opening an evaluation will close any current evaluation. To perform several evaluations at the same time, start additional instances of the software.*

Evaluation items

Evaluation is carried out in *evaluation items*. There are five basic item types (see also *Section 3.1 Overview of the Home workspace, on page 34*):

Item name	Description
Sensorgram	Used for sensorgram display and presentation.
Plot	Used for plot-based evaluation such as screening and ranking, and for PLA and EC_{50} determinations.
Concentration	Used for evaluation of concentration measurements.
Epitope binning	Used for evaluation of epitope binning experiments.
Kinetics and affinity [1]	Used for evaluation of interaction kinetics and affinity.

[1] Items are provided for evaluation of kinetics and affinity separately as well as in combination.

An evaluation session may hold any number of items of each type.

Initial display

The initial display when a new evaluation is created is determined by the content of the method according to the table below:

If the evaluation method contains...	Initial display
Solvent correction	The solvent correction workspace will be displayed first. Any remaining items in the method are created when solvent correction is applied or cancelled.
Evaluation items	The method definition defines which evaluation item should be displayed first (see *Chapter 11 Evaluation methods, on page 280*).
Neither solvent correction nor evaluation items	The ***Home*** workspace is displayed.

Note: *If data is from a Biacore T200 or Biacore S200 system and contains controls, the **Variables** table is displayed first.*

2.3 Evaluation workflow

Introduction

Runs are always opened for evaluation together with an evaluation method. When the method is appropriate to the purpose of the experiment, most or all of the evaluation procedure will be accomplished automatically (see *Chapter 11 Evaluation methods, on page 280*).

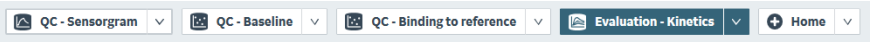
This section summarizes the general workflow for further evaluation.

Workflow summary

Stage	Description
1	Open the data to be evaluated.
2	Prepare the data as necessary by setting channel or flow cell status, adding or editing variables, creating report points, and applying solvent correction.
3	Create evaluation items as required. Use the ***Select sensorgrams*** panel if you want to change which sensorgrams are included in the evaluation (see *Workspace settings, on page 30*).
4	Create an evaluation method if you want to apply the same evaluation to other data sets.
5	Save and export the results as required.

Evaluation navigation bar

Created evaluation items are displayed in the evaluation navigation bar, above the evaluation workspace. Click on an item tab to navigate to that item, or click the ***Home*** tab to navigate to the home workspace.

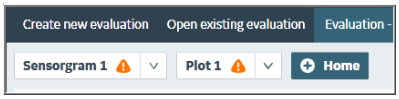


The drop-down menu on each item holds options to ***Rename***, ***Clone***, or ***Remove***. When the item is cloned, an exact copy of the item is created. The cloned item is independent and does not maintain synchronization with the original item. New items can be created via the drop-down menu on the ***Home*** tab.

Functions for settings and preparations, creation of evaluation method and result export are displayed as a tab to the far right in the navigation bar. The tab displays the latest active function.

Updating items

Whenever changes are made to evaluation settings that may affect existing items (for example, changes to a report point definition or global exclusion of sensorgrams), existing items may be flagged with a warning symbol on the item tab if there is a risk that the item may be affected by the change. Some items may be updated directly.



Flagged items are recalculated when they are reselected. Examine the updated items in relation to the changed settings to determine what has been changed (for example, if a report point used in a plot is deleted, the plot will be updated to use a different report point).

Note: *Updates will be applied automatically when the evaluation is saved or exported. Examine all items that are flagged with the warning symbol for unexpected changes before saving or exporting the evaluation.*

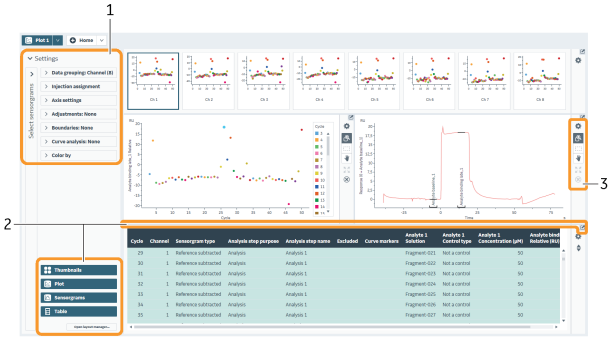
2.4 Evaluation settings

Introduction

Settings in the evaluation workspace are controlled in general at three levels:

- The ***Settings*** panel at the left of the workspace controls settings that apply to the whole evaluation item.
- The layout of the workspace is controlled by workspace layout functions.
- The panel toolbar at the right-hand side of each panel in an evaluation item controls settings that are applied to the panel or its contents.

Note: *Panels within an evaluation item provide different views of the same data. Some settings that are applied to selected contents of a panel will also affect the contents of other panels.*



Part	Function
1	Workspace settings
2	Workspace layout functions
3	Panel toolbar

Workspace settings

The main features of workspace settings are described in the table below.

Setting	Description
Settings	Click the ***Settings*** header to expand or collapse the ***Settings*** panel. Details of the settings are specific to different evaluation items, and are described in the respective evaluation item chapters.
Select sensorgrams	Click the ***Select sensorgrams*** header to expand or collapse the ***Select sensorgrams*** filter. Use ***Select sensorgrams*** to choose which sensorgrams to include in the evaluation. Add or remove checkmarks for the filters as required.

Workspace layout functions

The layout of the workspace can be customized, to increase focus on the elements that are most relevant for the current evaluation.

Layout function	Description
Panel buttons	Use the buttons at the bottom of the ***Settings*** panel to show or hide panels in the evaluation item. ***Note:*** *When thumbnails are hidden, they are replaced by buttons which allow the data groups to be selected even when the thumbnail content is not visible.*
Divider lines	The size of visible panels can be adjusted. Drag the divider lines between panels to adjust the panels' width or height.
Layout manager	Click ***Open layout manager*** to access a tool for rearrangement of panels in the evaluation item. Drag and drop the panels in the grid to their desired positions. Empty positions in the grid are ignored. The button ***Default layout*** resets the layout settings to those of a newly created item, in terms of panel visibility (show/hide), position and size. Click ***Apply and close*** to apply changes.

Layout function	Description
Panel in new window	Click the  symbol at the top of a panel toolbar to open a copy of the panel in a new window. The new window maintains synchronization with the rest of the evaluation item and can for example be moved to a second monitor, if available.

General panel toolbar settings

Settings that are common to several different panels are described in the table below. Settings that are specific to certain panels are described in the chapters dealing with the respective evaluation items.

Setting	Description
 ***Settings***	Specific to different panel types and evaluation items. Described in the respective evaluation item chapters.
 ***Zoom mode***	Enables display zooming in the panel. Drag with the mouse to enlarge a selected portion of the panel display. Double-click in the panel to restore the previous zoom level.
 ***Select area mode***	Enables selection by dragging over an area with the mouse. All curves or points that are partially or wholly enclosed in the area will be selected.
 ***Pan mode***	Enables panning.
 ***Zoom out max***	Restores the display to the default zoom factor and panning position.
 ***Deselect all***	Deselects all selected points or curves in the panel.

Copying panel content

Content from most panels can be copied to the Windows clipboard using the ***Copy*** options from the right-click menu.

Panel type	Copy function
Graphical charts	The chart is copied at the current zoom setting to the clipboard as a graphical object. The ***Copy graph*** option copies the chart as displayed. Most charts can also be copied as ***small***, ***medium*** and ***large*** images that use fixed proportions.
Tables	Selected rows are copied to the clipboard in tab-separated format. The table header row is included in the copied data.

In addition, graphical charts can be saved in Enhanced Metafile (`.emf`) or Portable Network Graphics (`.png`) format using ***Save as*** from the right-click menu. Curve coordinates from applicable charts (e.g., sensorgrams and affinity plots) can also be exported as numerical data in tab-separated text format, using ***Export curves*** from the right-click menu.

3 Home workspace

About this chapter

This chapter describes the ***Settings and preparation***, ***After evaluation***, and ***Export to*** functions in the ***Home*** workspace of the Evaluation Software. ***New evaluation items*** are described in the following chapters.

In this chapter

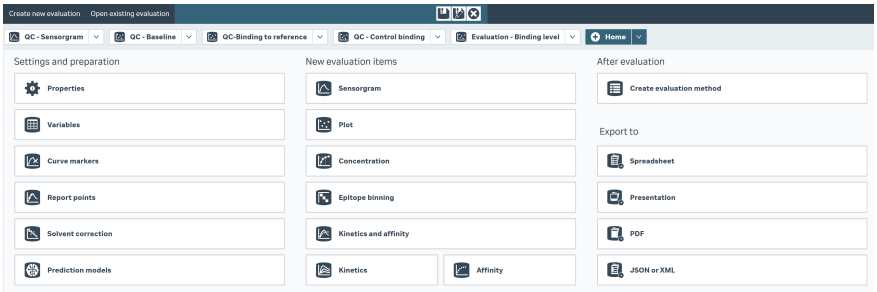
3.1 Overview of the Home workspace

Introduction

This section gives an overview of the ***Home*** workspace. The various tools are described in the sections that follow.

Overview of evaluation tools

Use the functions in the ***Home*** workspace for examining and modifying data properties, creating evaluation items, exporting evaluation results and saving evaluation methods. The table below gives a brief overview of the functions. More details are provided in their respective chapters.



Tool name	Description
Settings and preparation	
Properties 	Displays the properties of the current data set, and allows certain properties relating to the sensor surface to be edited for the evaluation session (see *Section 3.2 Properties, on page 37*).
Variables 	Displays a table of variable values. The values can be edited and user-defined variables can be added (see *Section 3.3 Variables, on page 40*).
Curve markers 	Manages curve markers, used for identifying sensorgrams and samples of interest. Curve markers are carried over between evaluation items so that samples of interest can be followed between items (see *Section 3.4 Curve markers, on page 44*.
Report points 	Defines report points for measuring the response at set times on the sensorgram (see *Section 3.5 Report points, on page 46*).

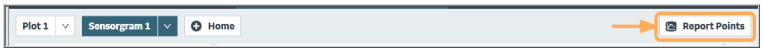
Tool name	Description
Solvent correction 	Evaluates and applies solvent correction (see *Chapter 4 Solvent correction principles and application, on page 57*).
Prediction models 	Lists and describes existing prediction models, and what application they are linked to. It also permits creation of new models (see *Section 3.6 Prediction models, on page 51*). This option is available if the ***Biacore Intelligent Analysis*** extension is active.
New evaluation items	
Sensorgram 	Creates a sensorgram item (see *Chapter 5 Sensorgram items, on page 71*).
Plot 	Creates a plot item (see *Chapter 6 Plot items, on page 89*).
Concentration 	Creates an item for evaluating concentration measurements (see *Chapter 7 Concentration items, on page 154*). This option is available if the ***Concentration & Potency*** extension is active.
Epitope binning 	Creates an item for evaluating epitope binning. This option is available if the ***Epitope Binning*** extension is active.
Kinetics and affinity 	Creates an item for evaluating affinity and kinetics in the same item[1] (see *Chapter 9 Kinetics and Affinity items, on page 207*).
Kinetics 	Creates an item for evaluating kinetics only[1] (see *Chapter 9 Kinetics and Affinity items, on page 207*).
Affinity 	Creates an item for evaluating affinity only[1] (see *Chapter 9 Kinetics and Affinity items, on page 207*).
After evaluation	

Tool name	Description
Create evaluation method 	Saves the settings and items in the current evaluation session as an evaluation method (see *Chapter 11 Evaluation methods, on page 280*). This option is not available for evaluation of regulated procedures, runs, and evaluations (***GxP*** extension).
Show audit trail 	Displays the audit trail for the current evaluation. This option is available when the ***GxP*** extension package is active, and the evaluation applies to regulated procedures, runs, and evaluations. See the *Biacore Insight GxP User Manual (29312548)* for more information.
Export to	
Spreadsheet 	Exports the results of the current evaluation session in Microsoft Excel format (see *Section 10.1 Export to spreadsheet, on page 271*).
Presentation 	Exports the results of the current evaluation session in Microsoft PowerPoint format (see *Section 10.2 Export to presentation or PDF, on page 273*).
PDF 	Exports the results of the current evaluation session to a PDF file (see *Section 10.2 Export to presentation or PDF, on page 273*).
JSON or XML 	Exports the results of the current evaluation session in JSON or XML export formats (see *Section 10.3 Export to JSON or XML, on page 277*). This option is available when the ***Data Integration*** extension is active.

[1] The evaluation purpose can be changed between kinetics, affinity and both within the item.

Note: *Changes in some evaluation tools are applied directly, while others show* ***Apply and close*** *and* ***Cancel*** *buttons at the bottom of the workspace. Make sure changes are applied if there is an option.*

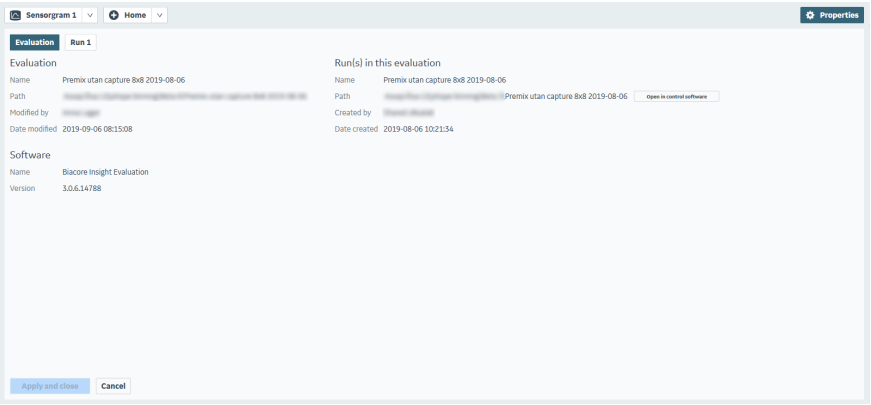
Tool workspaces remain open until actively closed. Open workspaces are represented by buttons at the right of the header bar. Use these buttons to return to the tool if the tool workspace is hidden.



3.2 Properties

Introduction

The ***Properties*** workspace displays properties of the evaluation session and the runs included in the session.



Evaluation properties

The ***Evaluation*** tab shows the following information:

Information	Description
Regulated procedure	Name and details of the evaluation session. This is only shown for regulated evaluations, available when the ***GxP*** extension is active.
Evaluation	Name and details of the evaluation session. For a new session that has not been saved, the session name is either the name of the run or ***Multiple runs***. This will be used as the default name when the session is saved.
Software	Software version used to create the evaluation session. For existing evaluations, this may differ from the version currently being run (the current version is shown in the ***Help*** →***About*** information).
Run(s) in this evaluation	Details of the run(s) in the evaluation session. Click ***Open in Control Software*** to open the run in Biacore Insight Control Software. Biacore Insight Control Software must be installed on the same computer. ***Note:*** *Runs imported from* ***.blr*** *result files cannot be opened in Biacore Insight Control Software.*

Information	Description
Applied signatures	Details of the signatures applied to the evaluation session. This is only shown for regulated evaluations, available when the ***GxP*** extension is active.

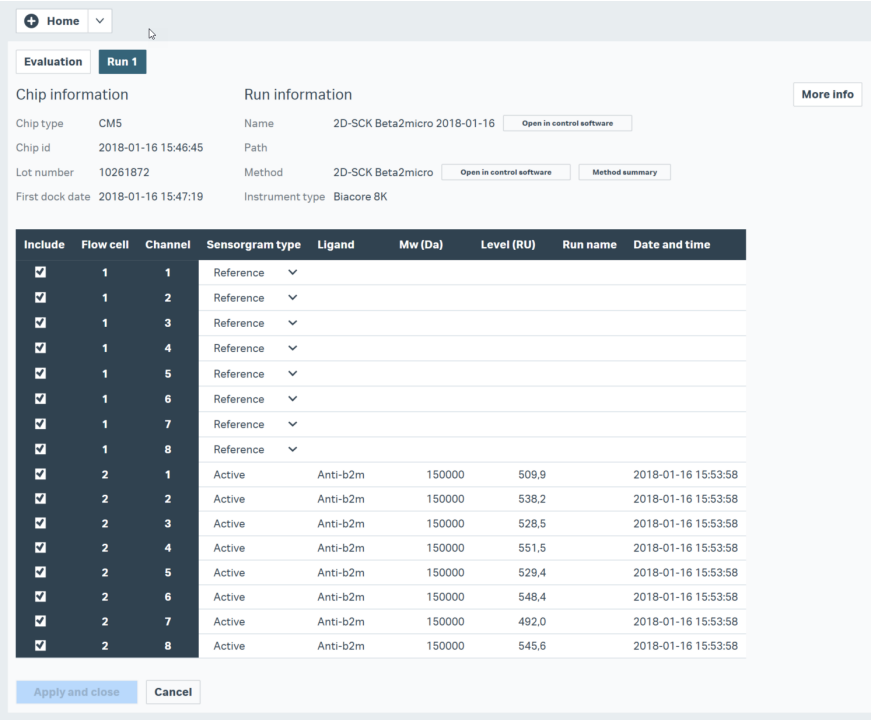
Run properties

One ***Run*** tab is created for each run represented in the data set.

Each ***Run*** tab shows the chip information and run information for the run together with channel (8-channel data) and flow cell usage details. The run and method can be opened in Biacore Insight Control Software. Click ***Method summary*** to open a summary of the method setup and settings, suitable for documentation purposes. The method summary is included in run properties in result exports.

Note: *It is not possible to open runs, methods or method summaries with data imported from* ***.blr*** *result files.*

The illustration below shows an example from evaluation of a Biacore 8K run. The detailed appearance may vary for runs from other Biacore systems.



Select ***More info*** to show additional details of the run information.

Channel and flow cell details can be edited as follows (provided that solvent correction has not been applied, see *Chapter 4 Solvent correction principles and application, on page 57*). Changes apply to the current evaluation only.

- Remove the checkmark from flow cells to exclude the flow cell result from the evaluation.
- Change the role of the flow cells if required. For 1-channel data, choose ***Custom*** to deviate from the settings from the run. The included flow cell furthest downstream is automatically set as ***Active***. Reference subtracted curves are generated based on instrument restrictions (see the *User Manual* for the appropriate Biacore system). For 8-channel data, ***Fc2*** is always Active. If ***Fc1*** is also set to ***Active***, reference-subtracted data will not be available.
- Edit the ligand name and molecular weight if required.

Changes made in the ***Run*** tab will not affect the values in the original saved run.

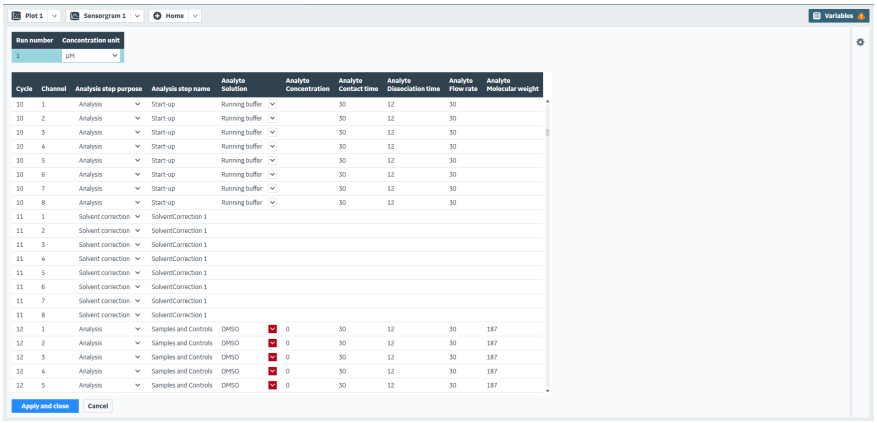
Note: *The workspace will remain open until you click **Apply and close** or **Cancel**, although it may be hidden by other workspaces. Changes will not be applied until you click **Apply and close**.*

3.3 Variables

Introduction

Variables are used in various aspects of evaluation, for sensorgram identification, analyte concentration, molecular weight adjustment, and so on. By default, the ***Variables*** workspace table does not show all available variables. See *Displaying table columns, on page 41* for instructions on how to show or hide variables in the table. Variable table settings are saved in evaluation methods.

All variables are available for use in the evaluation, regardless of whether they are shown in the table.



Variables may be derived from three sources:

- General variables from the run, such as cycle and channel numbers, step names and purposes, etc.
- Parameters that are defined as variables in the method used for the run.
- User-defined variables created in the evaluation session.

Note: *The workspace will remain open until you click **Apply and close** or **Cancel**, although it may be hidden by other workspaces. Changes will not be applied until you click **Apply and close**.*

Editing variable values

To change variable values, simply type the new value in the appropriate place in the table. For variables with a fixed set of possible values, such as ***Analysis step purpose***, choose the value from the drop-down list.

By default, the ***Analysis step purpose*** is set to ***Analysis*** for all cycles in interactive runs.

To change the units for concentration variables, change the ***Concentration unit*** setting. This changes the unit but not the numerical value. If the evaluation session includes data from several runs, the concentration units can be changed independently for each run.

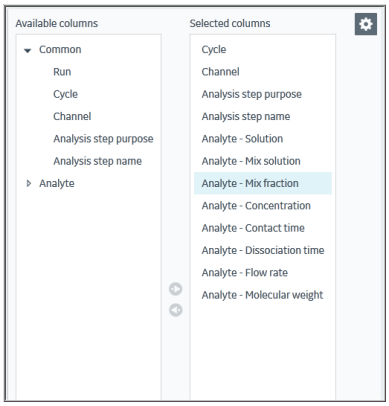
Note: *The concentration units affect only predefined concentration variables in the run method. User-defined variables defined in either the run method or the evaluation are not affected, even if they are used to hold concentration values.*

Select the sample status indicator in the ***Analyte solution*** column for injected solutions to define control and blank samples. Sample status of Dual and Poly command data is set for each sub-injection and sample. Changing the status of one instance of a sample will change all instances with the same solution name in the run. Other runs in the same evaluation session are not affected.

Displaying table columns

Follow the steps below to add or remove columns from the table.

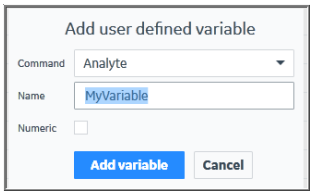
Note: *Table columns are added and removed for display purposes only. All variables are available for evaluation purposes, regardless of whether they are shown in the table.*

Step	Action
1	Click ***Table settings*** on the toolbar at the right of the workspace. *Result:* Lists of available and selected columns are displayed. 
2	Select a variable and use the arrow buttons to move it between the lists as required. Variables in the ***Available columns*** list are grouped according to source. To add all variables in a group to the table in one operation, select the group header in the left column.

Step	Action
	Note: *Variables remain in the **Available columns** list when they are added to **Selected columns**. You can however only add one instance of each variable to the **Selected columns** list.*
3	Click ***Apply and close*** to save the changes.

Adding user defined variables

Follow the steps below to add user defined variables to the evaluation.

Step	Action
1	Click ***Table settings*** on the toolbar at the right of the workspace.
2	Select ***Add*** at the bottom of the pane. *Result:* The ***Add user defined variable*** dialog opens. 
3	Select the ***Command*** (as set up in the analysis method) with which the variable will be associated.
4	Provide a name for the variable. ***Note:*** *The variable name must be unique within the command. Variables can however have the same name if they are associated with different commands.*
5	Select ***Numeric*** if the variable should only accept numerical values.
6	Select ***Add variable***. *Result:* The variable is created and is automatically displayed in the table.

Deleting user defined variables

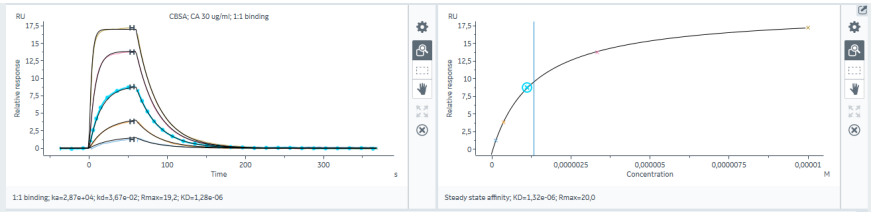
Follow the steps below to delete a user defined variable. You cannot delete a system defined variable, and you cannot delete a user defined variable that is not displayed in the table.

Step	Action
1	Click ***Table settings*** on the toolbar at the right of the workspace.
2	Select the variable in the ***Selected columns*** list.
3	Click ***Delete*** at the bottom of the ***Table settings*** panel.

3.4 Curve markers

Introduction

Curve markers are used to track interactions of particular interest through a complete evaluation. Curve markers can be set on sensorgrams, plot points or table rows in any evaluation item, and are shown in tool tips, tables and graphical presentations in all items.



- Sensorgrams with markers are shown with a series of points along the sensorgram.

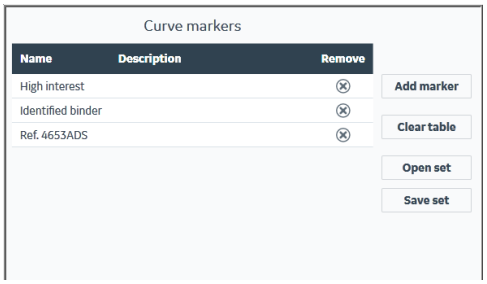
  Note: *The points do not represent measurement points.*

- In plots, points derived from sensorgrams with markers are circled. If a marked point is also selected, the point is shown in blue, larger than selected unmarked points.

Curve marker definitions may be saved as marker sets for use in multiple evaluations. Marker definitions are also saved in evaluation methods.

Defining curve markers

To define curve markers, choose either ***Curve markers*** in the ***Home*** workspace or ***Edit markers*** from any of the panel settings in an evaluation item. These alternatives are equivalent.



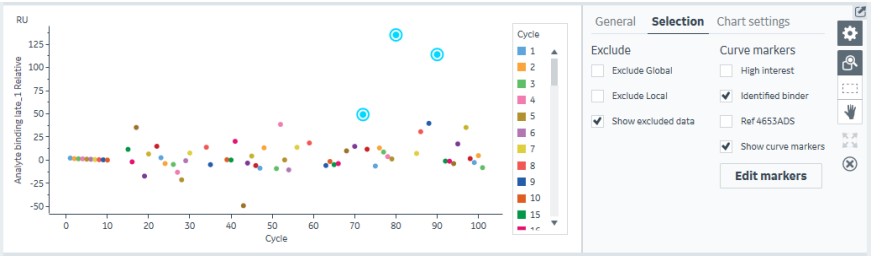
Choose ***Add marker*** and enter a marker name and description to define a new marker. The name must be unique within the evaluation session, and may not exceed 30 characters. Markers are identified by name in evaluation item displays: the description is only visible in the curve marker definition table.

Note: *A predefined curve marker named* ***Outside solvent correction range*** *is used to identify sensorgrams that lie partially or wholly outside the solvent correction range (see* *Section 4.4 Assessing solvent correction quality, on page 69**).*

Applying curve markers

To apply a curve marker in an evaluation item panel (plot, sensorgram, heat map, or table), select the target and choose ***Curve markers*** from the panel settings. Select the marker you want to apply. The marker will be applied to all selected targets in the panel, and will be shown in all evaluation item panels including thumbnails.

Derived sensorgrams and points inherit any curve markers from the source data (for example, blank subtracted sensorgrams inherit any curve markers from the unsubtracted sensorgram and the blank sensorgram).



Alternatively, right-click in the panel and choose the marker from the right-click menu.

Note: *It does not matter where you right-click in sensorgram or plot panels. However, in table panels, right-clicking on an unselected row automatically selects the row.*

Editing curve markers

Curve marker definitions cannot be edited. If you want to change the name or description for a marker, the marker must be deleted and a new marker created.

Curve marker sets

Curve marker sets can be saved and opened in a different evaluation session, so that the same curve markers can be used in multiple evaluation sessions without the need to recreate the markers individually in each session. Curve marker sets can either replace or be appended to any current curve markers in the evaluation session. When curve marker sets are appended to existing definitions, any naming conflicts are resolved by adding asterisks (*) to conflicting appended names.

Note: *All saved sets are available to all users.*

3.5 Report points

Introduction

Report points provide a measure of the response at specific time points in a sensorgram. The response is calculated as the median response within a short window (usually 5 s) centered on the specified report point time, and may be calculated relative to another report point defined as the baseline.

Note: *Report points in the system-specific software for Biacore T200 and Biacore S200 are calculated as the average response within the window. The precise response value for report points with the same position and window may consequently differ between evaluation in Biacore Insight Evaluation software and the system-specific software.*

The time for a report point is usually specified in relation to the beginning or end of an injection. For example, analyte binding is typically measured as report points such as ***Binding early*** set a few seconds after the start of the injection and ***Stability late*** a few seconds before the end of the dissociation. Times may also be set in relation to the beginning or end of the cycle.

The ***Report points*** workspace lists the report points in the data set. Report points are associated with injection types. Names are presented in a generic form in the workspace (for example, ***Analyte binding early***). Individual report points in the data are given a serial number suffix to distinguish between multiple injections of the same type in the same cycle (for example, ***Analyte binding early_1***).

A set of default report points is automatically created in the Evaluation Software when run results are opened, determined by the commands used in the run(s). Settings for these report points can be edited and custom report points can also be created.

Note: *Report points may be set at run time for some Biacore instruments, and are saved in the result file (**.blr**). These report points are ignored when the result file is imported to Biacore Insight Evaluation software.*

All report point definitions are saved in evaluation methods.

Note: *The workspace will remain open until you click **Apply and close** or **Cancel**, although it may be hidden by other workspaces.*

Predefined report points

Predefined report point names are constructed by combining the method command name with the report point, see the table below.

Command	Report point	Default position	Examples
Capture Enhance-ment	baseline	5 s before injection start	• Enhancement baseline • Enhancement level
	level	25 s after injection end	
Regenera-tion	baseline	5 s before injection start	• Regeneration level
	level	10 s after injection end	
A-B-A[1] Analyte C-O General SCK	baseline	5 s before injection start	• Analyte binding late • A-B-A baseline • General baseline
	binding early	6 s after injection start	
	binding late	5 s before injection end	
	stability early	5 s after injection end	
	stability late	5 s before dissociation end	
Dual	baseline	5 s before injection start	• Dual B binding late
	A binding early	6 s after injection start	
	A binding late	5 s before injection end	
	B binding early	6 s after injection start	
	B binding late	5 s before injection end	
	stability early	5 s after injection end	
	stability late	5 s before dissociation end	
Poly	baseline	5 s before injection start	• Poly C binding late
	A binding early	6 s after injection start	
	A binding late	5 s before injection end	
	B binding early	6 s after injection start	
	B binding late	5 s before injection end	

Command	Report point	Default position	Examples
	C binding early	6 s after injection start	
	C binding late	5 s before injection end	
	D binding early	6 s after injection start	
	D binding late	5 s before injection end	
	E binding early	6 s after injection start	
	E binding late	5 s before injection end	
	stability early	5 s after injection end	
	stability late	5 s before dissociation end	
Solvent	baseline	10 s before injection start	• Solvent level
	level	10 s before injection end	

[1] Report points for A-B-A commands are placed relative to the sample injection, not the injection of flanking solution.

Note: *Report point names are case-sensitive.*

Note: *Both baseline and response report points are assigned to each command in the run, so that relative response values within a command are calculated with reference to the baseline at the beginning of the command. Use calculated columns (see Section 6.8 Calculated columns, on page 148) if you want to calculate responses relative to other report points.*

Creating custom report points

Follow the steps below to create new report points:

Step	Action
1	Open the ***Report points*** workspace.
2	Select ***Add definition***.
3	Enter values for the settings. See *Report point settings, on page 49* for details. ***Note:*** *If you set the **Baseline** property for the new report point, relative response values for existing report points later in the cycle may change.*
4	Select ***Save***.

Report point settings

Details of settings for custom report points are given below:

Setting	Description
Name	Name of the report point. The name must be unique within the evaluation session. Predefined report point names (see *Predefined report points, on page 47*) may not be used even if the predefined report point is not included in the evaluation session.
Window size	Report point window in seconds. This may be any value between 1 and 35. The default value is 5. ***Note:*** *Report point values are calculated as the median response value in the report point window.*
Positioned	The time and event relative to which the report point is placed. Report points that are positioned before the start or after the end of the sensorgram cannot be used in the evaluation. ***Note:*** *Do not position report points far away from events so that they lose their relevance to the event, or so close to an event so that the report point window overlaps the event itself.*
of injection	The injection with which the report point will be associated. (Not shown if the event is ***cycle start*** or ***cycle end***.)
Baseline	Select to set the ***Baseline*** property for the report point. The relative response of a ***Baseline*** point is always zero. Relative responses for other report points are calculated relative to the nearest preceding ***Baseline*** point.

Editing report point settings

Follow the steps below to edit report point settings:

Step	Action
1	Open the ***Report points*** workspace.
2	Click ***Edit report point definition*** in the ***Actions*** column for the report point you want to edit.
3	Edit the settings as required. See *Report point settings, on page 49* for details. ***Note:*** *If you change the **Baseline** property for the report point, relative response values for existing report points later in the cycle will change.*
4	Select ***Save***.

To remove report points, select ***Remove report point definition*** in the ***Actions*** column.

Note: *Report points for solvent correction cannot be changed in this workspace. Adjust report point settings for solvent correction in the solvent correction workspace.*

3.6 Prediction models

Introduction

The ***Prediction models*** workspace is available with the ***Biacore Intelligent Analysis*** extension.

Prediction models interpret specific features derived from sensorgrams.

A model can be trained by using predefined classifications. A trained model can automatically predict the result of the application to which it has been applied. A user can continue to train an existing model, with the result becoming a new model version. Alternatively, a user can create a new model from an existing model. For more information, see *Section 6.6 Biacore Intelligent Analysis for binder prediction, on page 131*

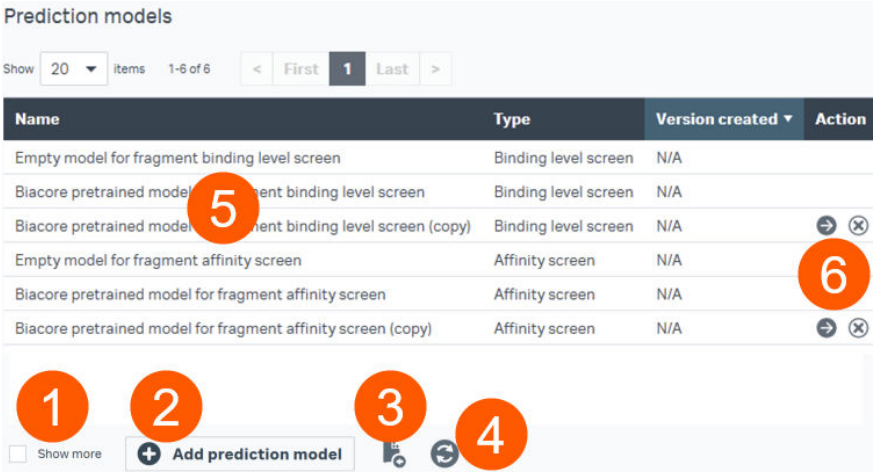
and *Section 9.6 Biacore Intelligent Analysis for affinity screen evaluation, on page 247*.

In this section

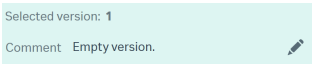
3.6.1 Panel description

The ***Prediction models*** panel shows available models, listed with columns for name, type, version created and action.

The user interface is described below:



	Button	Description
1	***Show more***	Click ***Show more*** to expand the prediction model panel.
2	***Add prediction model***	Click ***Add prediction model*** to add a prediction model. Enter a model name and a model description. Use the drop-down lists to select a model and version as the base for the new model. (Optional) Below ***Edit classifications***, click on a cell and enter a new name. (Optional) Edit a purpose by clicking on the pen icon: This is only possible when the new model is based on one of the empty models included with the Biacore Intelligent Analysis extension. ***Note:*** *A change of classification name or classification purpose does not affect how the model does the prediction. Instead, this requires the model to be trained.*
3		Click the ***Import icon*** to import all versions of the model to the database.
4		Click the ***Refresh icon*** to refresh/update the database.

	Button	Description
5		Select a model in the table to display the ***Versions*** table, which shows the version history and provides the possibility to remove a specific version. Select a version in the ***Versions*** table to display comments related to the version. 
6		Click ***Export*** in the ***Action*** column to export all versions of the prediction model to a file.
		Click ***Remove*** to remove all versions of the model.

Note: *To completely remove a model version, that version and all subsequent versions must be deleted. Version 1 of a model can only be removed by deleting the entire model.*

Note: *It is not permitted to remove models and versions used in* ***Procedures*** *(available in the* ***GxP*** *extension).*

3.6.2 Empty models and Biacore pretrained models

The ***Empty model*** and the Biacore pretrained models only have one version: ***version 1***. It is not possible to rename, remove, export, add new versions or change classification names or purposes for these models or model versions.

The ***Empty model*** and the Biacore pretrained models are only available from ***Prediction Models***, not in the evaluation item.

Pretrained models for fragment binding level screen and fragment affinity screen are available for prediction and training in their respective evaluation items.

Tip: *The **Prediction models** can be selected as a base when adding a new prediction model with a new name.*

3.7 Solvent correction

About this tool

Solvent correction adjusts response values for small artefacts introduced by the use of organic solvents in samples. The adjustment is only relevant in work with low molecular weight analytes. The ***Solvent correction*** tool is described in a separate chapter (*Chapter 4 Solvent correction principles and application, on page 57*).

3.8 After evaluation

Overview

This group of tools includes the following functions, typically used when the evaluation is complete:

- ***Create evaluation method***. This creates a custom evaluation method based on the current evaluation session. See *Creating custom evaluation methods, on page 280* for details.
- ***Spreadsheet***. This exports a Microsoft Excel file, suitable for archiving or further processing in third party applications. See *Section 10.1 Export to spreadsheet, on page 271* for details.
- ***Presentation***. This exports a Microsoft PowerPoint file, suitable for presentation purposes. See *Section 10.2 Export to presentation or PDF, on page 273* for details.
- ***PDF***. This exports a PDF file, suitable for documentation purposes. See *Section 10.2 Export to presentation or PDF, on page 273* for details.
- ***JSON or XML***. This exports a JSON file or an XML file. This tool is available when the ***Data Integration*** extension is active. See *Section 10.3 Export to JSON or XML, on page 277* for details.

4 Solvent correction principles and application

About this chapter

Solvent correction adjusts reference-subtracted responses for small artefacts that can be introduced by variations in the bulk refractive index between samples. The correction is only relevant when variations in the bulk refractive index are of a similar order of magnitude to the sample responses. This situation arises in work with small organic molecules that give intrinsically low response values and often require organic solvents with a high bulk refractive index such as dimethyl sulfoxide (DMSO) to maintain solubility.

Solvent correction is only relevant in runs using low molecular weight analytes that require organic solvents to maintain solubility.

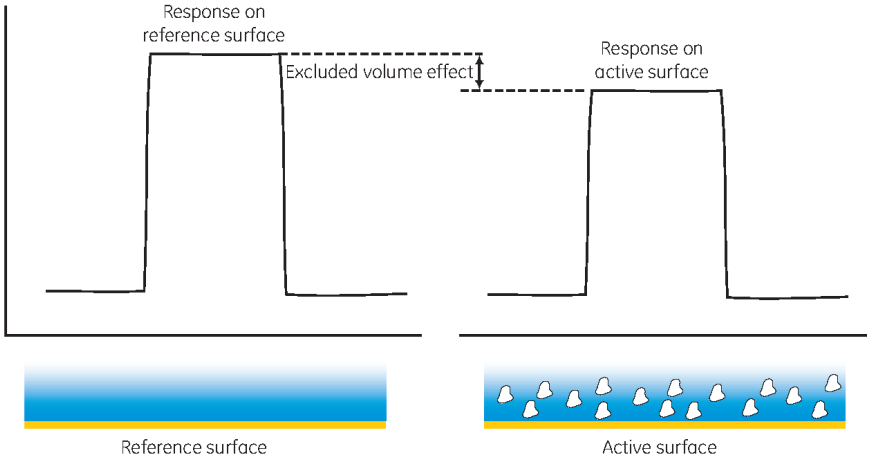
This chapter describes the principles and application of solvent correction in the Evaluation Software.

In this chapter

4.1 What is solvent correction?

Background

The need for solvent correction arises when the amount of ligand on the active surface is high compared with the reference, and the bulk refractive index contribution of the solvent is high compared with the expected analyte response. Bulk solution is excluded from the volume occupied by ligand on the active surface, so that bulk contributions on the active and reference surface will be slightly different, introducing a small error in the reference-subtracted response. This is illustrated schematically below.



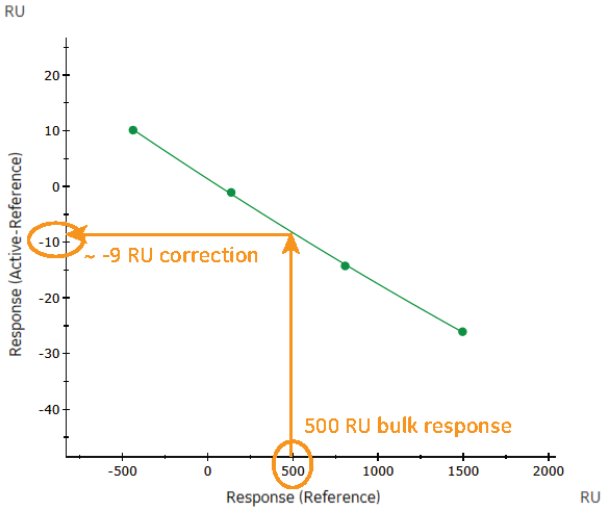
As long as the refractive index of the samples is constant, the error in the reference-subtracted response is also constant and may be ignored for practical purposes. However, if the refractive index of the samples varies, the magnitude of the error will also vary.

Addition of 1% DMSO to the buffer gives a bulk response of about 1200 RU, so that small variations in the DMSO content lead to significant variations in the bulk response, in relation to the expected response from low molecular weight samples (which may be as little as 5 to 10 RU). Such variations are difficult to avoid in the preparation of samples such as fragments and drug candidates for screening applications.

Principle of solvent correction

Solvent correction is determined by injecting a series of blank samples containing a range of solvent concentrations over the active and reference surfaces. A plot of the relative reference-subtracted response on the active surface against the absolute response on the reference surface calibrates the error in reference subtraction against the bulk contribution. This calibration is then used to correct the measured sample responses.

In the schematic illustration below, samples that gave a response of 500 RU on the reference surface would be corrected by about -9 RU on the reference-subtracted response.



Each solvent correction cycle generates a separate curve for each reference subtracted sensorgram. In practice, this results in one curve for each channel and solvent correction cycle in Biacore 8 series instruments. Solvent correction is only performed using correction data from the same pair of flow cells as the sample.

It is recommended that solvent correction cycles are run at the beginning and end of a run and at regular intervals during the run. In this way, any given sample cycle will lie between two solvent correction cycles. The correction factor for a given sample cycle is determined by interpolation between the curves from preceding and following correction cycles, to compensate for any drift in the solvent correction factors during the course of the run.

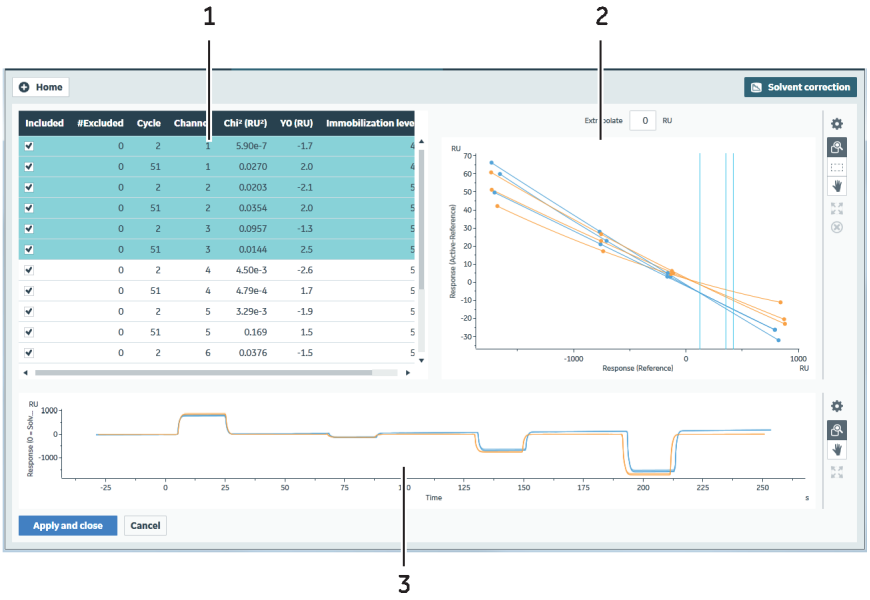
Note: *Interpolation between solvent correction curves is not performed in system-specific evaluation software for Biacore T200 and Biacore S200. Solvent correction factors can consequently differ slightly for the same data evaluated in Biacore Insight Evaluation software and system-specific software.*

4.2 The Solvent correction workspace

In this section

4.2.1 Introduction

Solvent correction is managed from a workspace with three panels:



Panel	Content	Description
1	Table	Lists numerical values for each solvent correction cycle in the data set. See *Section 4.2.2 Table panel, on page 62* for more details.
2	Correction curves	Shows the curves currently selected in the table. See *Section 4.2.3 Curves panel, on page 63* for more details.
3	Sensorgrams	Shows sensorgrams for the currently displayed correction curves. See *Section 4.2.4 Sensorgrams panel, on page 65* for more details.

4.2.2 Table panel

Panel description

Each row in the table panel corresponds to one solvent correction curve.

Select or deselect rows in the table to show or hide the corresponding correction curves in the ***Curves*** panel.

Remove the checkmark from the ***Included*** column to exclude curves from being used when solvent correction is applied. Excluded solvent correction curves are shown in light gray in the ***Curves*** panel.

Note: *Hiding a curve by deselecting the table row does not affect the* ***Include*** *status.*

The parameters ***Chi^2*** and ***Y0*** in the table represent respectively the closeness of curve fit and the curve intercept on the y-axis.

4.2.3 Curves panel

Panel description

The ***Curves*** panel shows all solvent correction curves by default. Deselect curves in the ***Table*** panel to hide them. Curves are fitted to the experimental points using a second-degree polynomial function.

Click on a point to select it. Alternatively, use the ***Area select*** tool from the toolbar (see *General panel toolbar settings, on page 31*) to select multiple points.

Outlying points on the solvent correction curves are detected and excluded automatically by the software. To exclude points manually, select the points, then right-click in the panel and choose ***Exclude selected***. Excluded points are shown as a gray ×.

To re-include manually and automatically excluded points, select the points and then right-click and choose ***Include selected***.

Vertical blue lines in the ***Curves*** panel indicate the range of report point values for samples affected by the displayed curves. Separate lines are shown for each reference. Sample responses that lie outside the range of the solvent correction curves will not be corrected, and will not appear in evaluation items that use corrected responses. This can create gaps in sensorgram display.

Enter a value for ***Extrapolate*** to extend the curves by the specified amount in both directions on the x-axis. Extrapolation is applied to all curves, regardless of whether they are currently displayed.

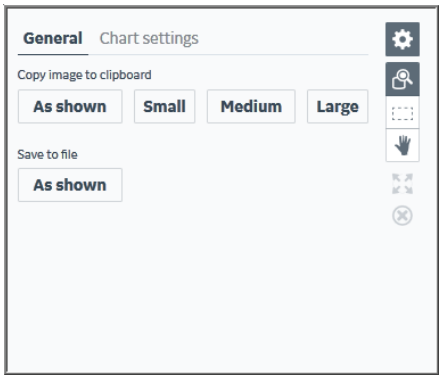
Note: *Extrapolation relies on the fitting parameters for the solvent correction curve, with no further justification from experimental data.*

Panel toolbar

Click ***Chart settings*** on the panel toolbar to access display settings for the panel.

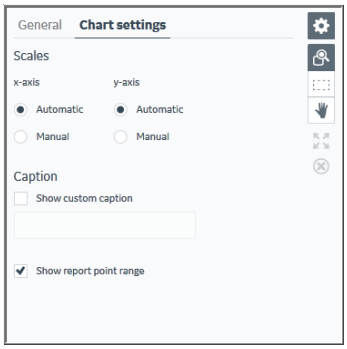
The separate tabs in the chart settings are described below. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.

General tab



Setting	Description
Copy image to clipboard	Copies the plot panel to the Windows clipboard. Choose one of three sizes or ***As shown***. ***Note:*** ***Small***, ***Medium*** *and* ***Large*** *copies use default image proportions.* ***As shown*** *retains the proportions as displayed on the screen.*
Save to file	Saves the sensorgram panel as an illustration in selectable format.

Chart settings tab



Setting	Description
Scales	Choose ***Automatic*** or ***Manual*** scales for the axes.
Caption	Includes a caption in the panel. Enter the caption text.
Show report point range	Check this option to show the range of sample report point responses in the ***Curves*** panel.

4.2.4 Sensorgrams panel

Panel description

The ***Sensorgrams*** panel shows the solvent correction cycles for the currently displayed correction curves. Report points are indicated for correction points that are selected in the ***Curves*** panel.

The position and window of solvent correction report points can be adjusted (see *Edit report point tab, on page 66*). Adjustments apply to all injections in solvent correction cycles.

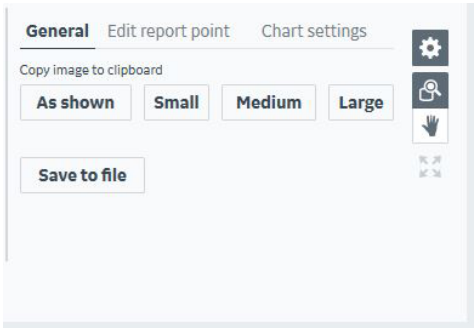
Note: *Sensorgrams cannot be selected in the* ***Sensorgrams*** *panel.*

Panel toolbar

Click ***Chart settings*** on the panel toolbar to access display settings for the panel.

The separate tabs in the chart settings are described below. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.

General tab



Setting	Description
Copy image to clipboard	Copies the plot panel to the Windows clipboard. Choose one of three sizes or ***As shown***. ***Note:*** ***Small***, ***Medium*** *and* ***Large*** *copies use default image proportions.* ***As shown*** *retains the proportions as displayed on the screen.*
Save to file	Saves the sensorgram panel as an illustration in selectable format.

Edit report point tab

Settings on this tab allow the placing of report points to be adjusted on the solvent correction sensorgrams. Adjustments apply to all injections in all solvent correction cycles. Select solvent correction points in the ***Curves*** panel to show the location in the ***Sensorgram*** panel. Solvent correction curves are recalculated whenever a report point setting is changed.

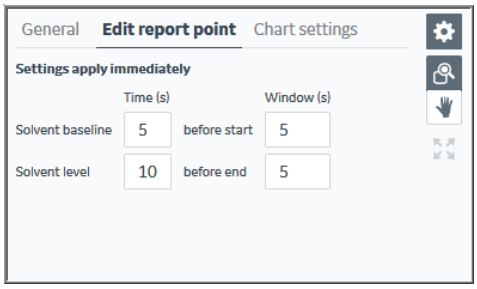
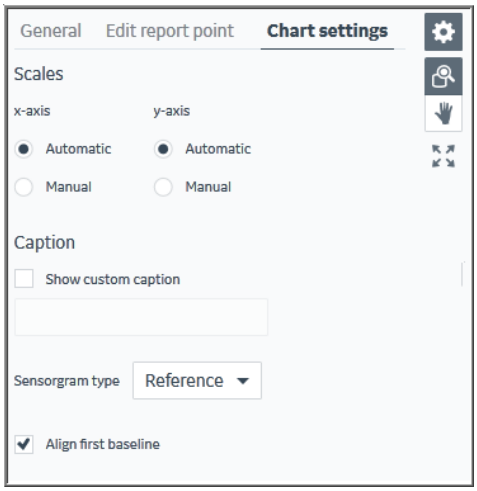


Chart settings tab



Setting	Description
Scales	Choose ***Automatic*** or ***Manual*** scales for the axes.
Caption	Includes a caption in the panel. Enter the caption text.
Sensorgram type	Choose whether to display the active, reference, or reference-subtracted sensorgrams.
Align first baseline	Check this option to align the baseline point for the first injection to zero time and response.

4.3 Applying solvent correction

Prerequisites

Applying solvent correction requires that solvent correction cycles have been included in the run (see the respective system *User Manual* for details). Once the correction has been applied, corrected data are used by default in evaluation items.

Procedure

Follow the steps below to apply solvent correction.

Note: *Each solvent correction curve must have at least 3 included points.*

Tip: *In data sets with many solvent correction curves, sorting the table by cycle or flow cell/channel can simplify selection and examination of curves.*

Step	Action
1	Select ***Solvent correction*** in the ***Tools*** workspace if the ***Solvent correction*** workspace is not opened by the evaluation method. *Result:* The ***Solvent correction*** workspace opens. All solvent correction sensorgrams and solvent correction curves are displayed initially.
2	To display selected solvent correction sensorgrams and curves, select the corresponding rows in the table. Use **Shift-click** and **Ctrl-click** to select multiple rows. ***Note:*** *Selecting rows for display does not affect the* ***Include*** *status for the curves.*
3	Check the solvent correction curves and sensorgrams for quality (see *Section 4.4 Assessing solvent correction quality, on page 69*). Exclude bad curves by removing the checkmark in the ***Included*** column of the table. Excluded curves are shown in gray. Exclude individual outliers that are not automatically removed as follows. **a.** Select the outlier(s), either by clicking on the points or using the ***Select area mode*** (see *General panel toolbar settings, on page 31*). **b.** Right-click in the curve plot area and choose ***Exclude selected***. Excluded points are shown with the symbol '×'.
4	Select ***Apply and close*** to apply the corrections, or ***Cancel*** to close the workspace without applying corrections.

Note: *Changes will not be applied until you click* ***Apply and close****. The workspace will remain open until you apply or cancel the corrections, although it may be hidden by other workspaces.*

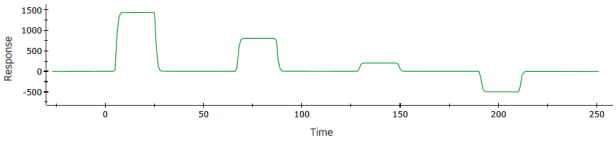
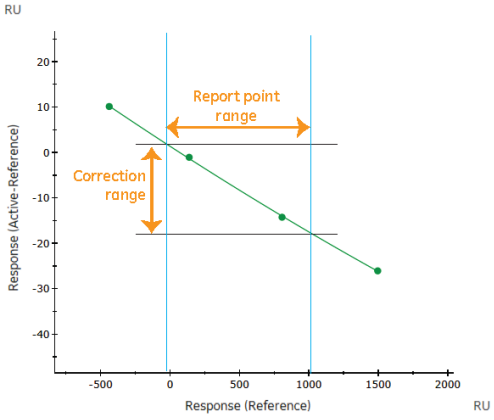
Solvent corrected data is used by default in all new items if it is available. Existing items are not affected.

Once solvent correction has been applied, you can open the workspace to view the curves and details, but you cannot make any changes to the solvent correction settings. Create a new evaluation if you want to change the solvent correction settings.

4.4 Assessing solvent correction quality

Quality criteria

Judge whether to use solvent corrected or uncorrected data in evaluation items according to the guidelines below.

Property	Recommendation
Solvent correction sensorgram quality	Check the appearance of the solvent correction sensorgrams on the active and reference surfaces. Each injection of solvent correction solution should give a "square-wave" response, positive or negative, with rapid transition to and from the baseline and essentially constant response during the injection (as illustrated below). If there are any disturbed injections, exclude the corresponding points from the solvent correction curves. 
Solvent correction curve range	The solvent correction curve should normally cover a response range of approximately -500 to +1500 RU on the x-axis (reference flow cell).
Correction range	The y-axis range of the curves between the report point range lines gives an indication of the range of solvent correction for report points, as indicated schematically below. Compare this with the range of measured response values to judge the effect of solvent correction on the data. 

Property	Recommendation
Curve quality	The solvent correction curves should be a reasonably close fit to the experimental points. As a rule of thumb, chi-square values should be below 2 RU. Exclude any isolated outlying points from the curves. ***Note:*** *Solvent correction curves with only 3 data points will fit the experimental data exactly and will have a chi-square value of 0.* Beware of using solvent corrected data if the correction curve does not fit the experimental points closely. Scatter in the correction points can distort the corrected responses.
Data point range	Sensorgrams with points that lie outside the solvent correction range will be assigned a curve marker ***Outside solvent correction range*** when correction is applied. For such sensorgrams, data points within the correction range will be corrected while those outside the range will be omitted from the sensorgram display and evaluation.

5 Sensorgram items

About this chapter

Sensorgram items display the data as sensorgrams for presentation and visual inspection purposes.

This chapter describes how to use ***Sensorgram*** items.

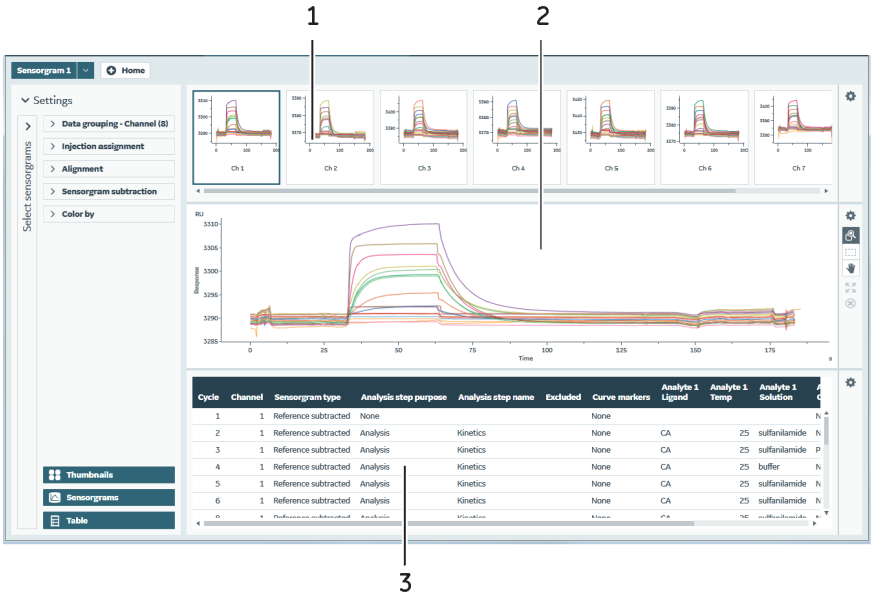
In this chapter

5.1 The Sensorgram workspace

In this section

5.1.1 Introduction

The ***Sensorgram*** item workspace holds up to 3 panels in addition to the ***Settings*** panel at the left of the workspace.



Panel	Content	Description
1	***Thumbnails***	Displays the data groups in the evaluation. See *Section 5.1.2 Thumbnails panel, on page 74* for details.
2	***Sensorgrams***	Displays the sensorgrams in selected groups. See *Section 5.1.3 Sensorgrams panel, on page 77* for details.
3	***Table***	Displays the details for the cycles in the selected groups. See *Section 5.1.4 Table panel, on page 83* for details.

Drag the panel borders to resize the panels.

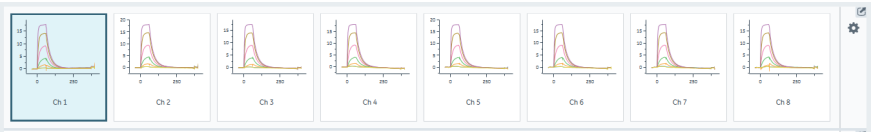
Use the buttons at the bottom of the ***Settings*** panel to show or hide selected panels.

The panels are described in more detail in the following subsections.

5.1.2 Thumbnails panel

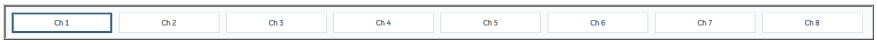
Panel description

The ***Thumbnails*** panel displays thumbnails of the sensorgram data grouped according to the ***Data grouping*** setting. You may need to scroll the panel display to see all thumbnails.



Select one or more thumbnails to display the contents in the ***Sensorgram*** and ***Table*** panels. Use **Shift-click** and **Ctrl-click** to select multiple thumbnails. Selected thumbnails are marked by a heavier border.

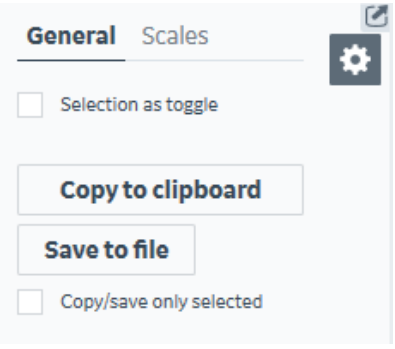
Deselect ***Thumbnails*** in the ***Settings*** panel at the left to collapse the thumbnails to buttons. Data groups can be selected with these buttons even when the thumbnail content is not displayed.



Panel toolbar

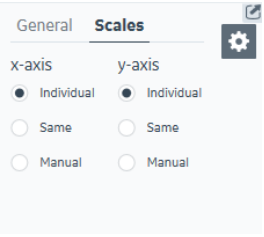
Click ⚙ ***Thumbnail settings*** on the panel toolbar to access display settings for the thumbnails. Settings are applied to all thumbnails.

General tab



Setting	Description
Selection as toggle	If this option is checked, clicking on a thumbnail will toggle between selected and deselected. When the option is not selected, clicking will only select the thumbnail. ***Note:*** *When the option is checked, using* **Shift-click** *will toggle the status of all thumbnails in the range covered.*
Copy to clipboard	Copies thumbnails to the Windows clipboard as a collection of graphical objects. The thumbnails can be pasted into programs that support pasting for collections of graphical objects. Check ***Copy/save only selected*** to copy only selected thumbnails. If this option is not checked, all thumbnails will be copied.
Save to file	Saves thumbnails as illustrations in ***.png*** (Portable Network Graphics) format. Each thumbnail is saved to a separate file. File names are constructed from the thumbnail label with an added serial number to prevent duplicate names. Check ***Copy/save only selected*** to save only selected thumbnails. If this option is not checked, all thumbnails will be saved.

Scales tab

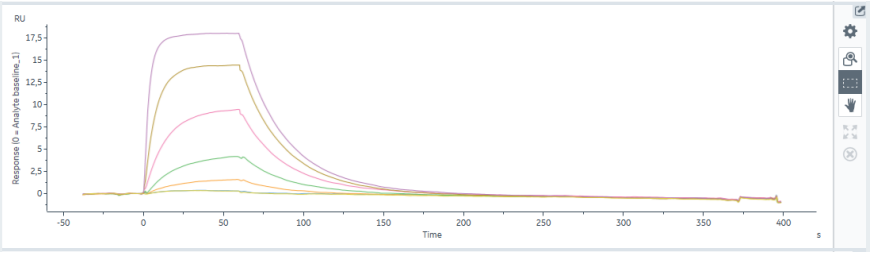


Setting	Description
x-axis ***y-axis***	Determines how the axes will be scaled in the thumbnails. • ***Individual*** scales each thumbnail separately. The scale is determined by the range of values in the thumbnail. • ***Same*** scales all thumbnails to the same scale, determined by the range of values in all thumbnails together. • ***Manual*** scales all thumbnails according to the specified minimum and maximum values.

5.1.3 Sensorgrams panel

Panel description

The ***Sensorgrams*** panel displays the sensorgrams in the currently selected thumbnail(s).



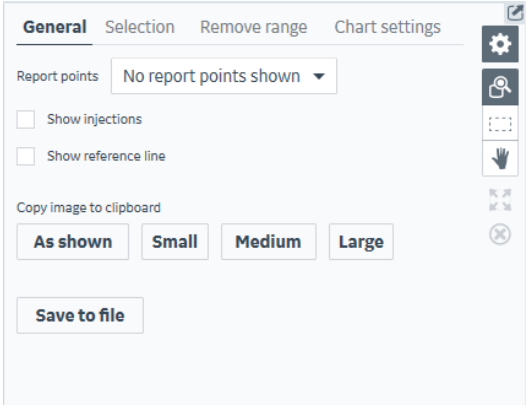
Individual sensorgrams can be selected in this panel. Click on a sensorgram to select/deselect it, or use the ***Area select*** mode to select multiple sensorgrams by dragging (see *General panel toolbar settings, on page 31*). Clicking on successive sensorgrams adds the sensorgrams to the selection. Selected sensorgrams are highlighted with a thicker blue line. Use ***Deselect all*** to deselect all selected points.

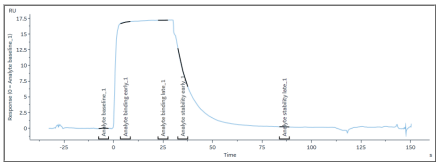
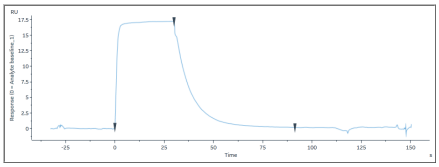
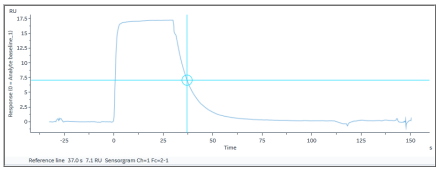
Panel toolbar

Click ***Chart settings*** on the panel toolbar to access display settings for the panel.

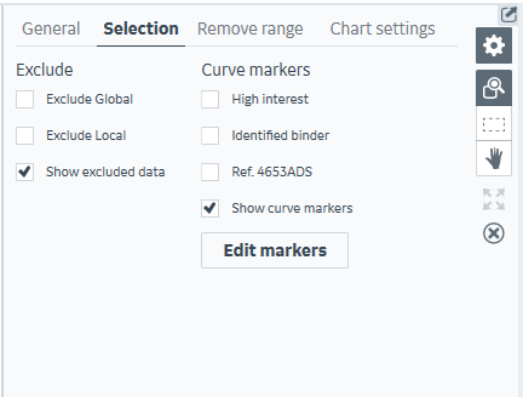
The separate tabs in the chart settings are described below. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.

General tab



Setting	Description
Report points	Choose whether to show report points, with or without markers and labels. 
Show injections	Check this option to show markers for the start and end of injections, including (where appropriate) the end of dissociation time. 
Show reference line	Check this option to show crosshairs on the sensorgram together with coordinate values for the crosshair position. Drag the vertical crosshair line to move the crosshairs. 
Copy image to clipboard	Copies the sensorgram panel to the Windows clipboard. Choose one of three sizes or ***As shown***. ***Note:*** ***Small***, ***Medium*** *and* ***Large*** *copies use default image proportions.* ***As shown*** *retains the proportions as displayed on the screen.*
Save to file	Saves the sensorgram panel as an illustration in selectable format.

Selection tab



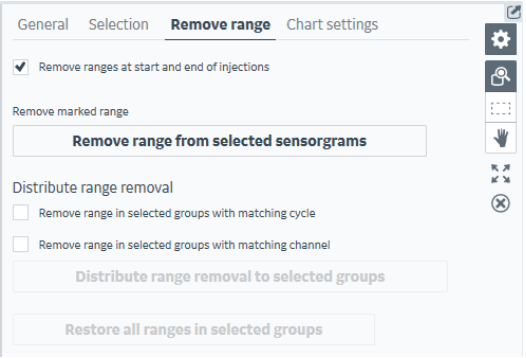
Note: *The curve markers shown here are examples, for illustration purposes only.*

Options on this tab apply to selected sensorgrams. See *Section 5.3 Selecting data in Sensorgram items, on page 88* for details on how to select sensorgrams.

Setting	Description
Exclude global	Check this option to exclude the selected sensorgrams from all evaluation items, including those already created. If excluded sensorgrams are selected, the option will be checked. Remove the checkmark to include the sensorgrams again. Excluding sensorgrams removes any sensorgram adjustments and/or table calculations that may have been applied.
Exclude local	Check this option to exclude the selected sensorgrams from the current evaluation item only. If excluded sensorgrams are selected, the option will be checked. Remove the checkmark to include the sensorgrams again.
Show excluded data	Check this option to show excluded data in the sensorgram panel. Excluded sensorgrams are shown as gray lines.
Curve markers	Check the required markers to apply the markers to the selected sensorgrams. See *Section 3.4 Curve markers, on page 44* for more details.
Show curve markers	Check this option to show curve markers in the sensorgram and thumbnail panels.

Setting	Description
Edit curve markers	Choose this option to manage curve markers. See *Section 3.4 Curve markers, on page 44* for details.

Remove range tab



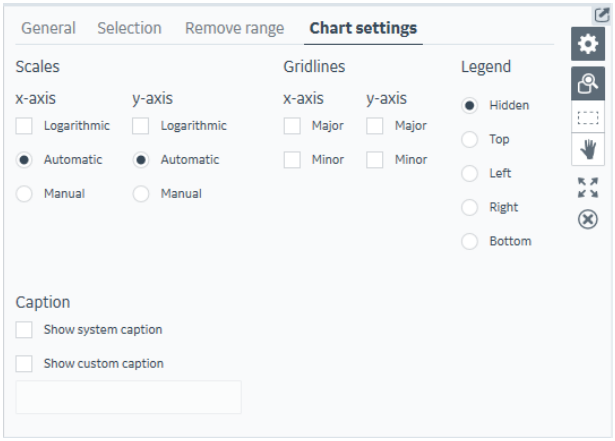
This action removes specified data ranges from selected sensorgrams. Drag the vertical markers to set the range that will be removed. Select one or more sensorgrams to activate the buttons.

Note: *Ranges are removed in the current evaluation item only.*

Setting	Description
Remove range at start & end of injections	When this option is checked, a fixed range (about 1 s) on either side of the sample injection start and stop events is removed, to avoid interference from disturbances that can occur at the injection event. ***Note:*** *This option affects all sensorgrams in all data series, regardless of selection.*
Remove range from selected sensorgrams	Click to remove the marked range from the selected sensorgrams. Only the selected sensorgrams in the current series will be affected.

Setting	Description
Remove range in selected series with matching cycle	Check this option to extend the selection to include sensorgrams with the same cycle number from all selected series. This option is primarily suitable for data with disturbances in multiple flow cells in the same cycle (1-channel data), or in parallel mode where different concentrations of the same sample are run in different channels in the same cycle (8-channel data).
Remove range in selected series with matching flow cell/ channel	Check this option to extend the selection to include sensorgrams in the same flow cell (1-channel data) or channel (8-channel data), from all selected series. Use this option primarily in serial mode where different concentrations of the same sample are run in different cycles in the same flow cell or channel.
Distribute range removal to selected series	Click to remove the marked range in accordance with the ***Distribute range removal*** setting.
Restore ranges for selected series	Click to restore removed ranges in the selected series. Removed data will be restored in all selected series, even if data was removed in several separate operations. ***Note:*** *You cannot restore ranges for individually selected sensorgrams.*

Chart settings tab



Setting	Description
Scales	Choose ***Automatic*** or ***Manual*** scales for the axes. For ***Manual*** scaling, enter minimum and maximum values. Choose ***Logarithmic*** to use a logarithmic axis scale. ***Note:*** *Zero or negative values cannot be displayed on a logarithmic scale.*
Gridlines	Choose whether to show major and/or minor gridlines. Gridline spacing is determined according to the scale range.
Legend	Displays a legend identifying sensorgrams by color. Choose the position of the legend in the panel.
Caption	Displays a caption in the panel. Choose whether to include the system caption and/or a custom caption with text as entered.

5.1.4 Table panel

Panel description

The table panel lists parameter values for all sensorgrams included in the sensorgram item. The currently selected sensorgram(s) are highlighted in blue.

Cycle	Channel	Sensorgram type	Analysis step purpose	Analysis step name	Excluded	Curve markers	Analyte 1 Solution	Analyte 1 Control type	Analyte 1 Concentration (µM)
14	1	Reference subtracted	Analysis	Positive control			Filibuvir	Positive control	0,25
27	1	Reference subtracted	Analysis	Positive control			Filibuvir	Positive control	0,25
40	1	Reference subtracted	Analysis	Positive control			Filibuvir	Positive control	0,25
54	1	Reference subtracted	Analysis	Positive control			Filibuvir	Positive control	0,25
14	2	Reference subtracted	Analysis	Positive control			Filibuvir	Positive control	0,25
27	2	Reference subtracted	Analysis	Positive control			Filibuvir	Positive control	0,25
40	2	Reference subtracted	Analysis	Positive control			Filibuvir	Positive control	0,25
54	2	Reference subtracted	Analysis	Positive control			Filibuvir	Positive control	0,25
14	3	Reference subtracted	Analysis	Positive control			Filibuvir	Positive control	0,25

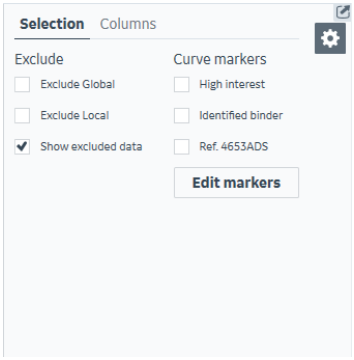
Click on a column header to sort the table by the content of that column.

Panel toolbar

Click ***Table settings*** on the panel toolbar to access display settings for the panel.

The separate tabs in the table settings are described below.

Selection tab

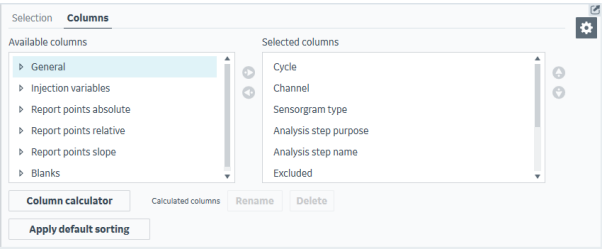


Note: *The curve markers shown here are examples, for illustration purposes only.*

Options on this tab apply to selected table rows. See *Section 5.3 Selecting data in Sensorgram items, on page 88* for details of how to select sensorgrams.

Setting	Description
Exclude global	Check this option to exclude the selected sensorgrams from all evaluation items, including those already created. If excluded sensorgrams are selected, the option will be checked. Remove the checkmark to include the sensorgrams again.
Exclude local	Check this option to exclude the selected sensorgrams from the current evaluation item only. If excluded sensorgrams are selected, the option will be checked. Remove the checkmark to include the sensorgrams again.
Show excluded data	Check this option to show excluded sensorgrams as gray lines in the sensorgram panel and thumbnails, and to include excluded data in the table. Excluded sensorgrams are identified in the table in the ***Excluded*** column. If this option is not checked, sensorgrams and corresponding table rows are hidden.
Curve markers	Check the required markers to apply the markers to the selected sensorgrams. See *Section 3.4 Curve markers, on page 44* for more details.
Edit markers	Choose this option to manage curve markers. See *Section 3.4 Curve markers, on page 44* for details.

Columns tab



Move columns between ***Available columns*** and ***Selected columns*** to control the information displayed in the table. Select a column in the ***Selected columns list*** and use the up and down arrows to change the column display order.

Click ***Column calculator*** to define table columns that hold results calculated from other column values, see *Section 6.8 Calculated columns, on page 148*.

Click ***Apply default sorting*** to restore the default sort order for table rows if you have changed the sort order.

Note: *Applying default sorting does not affect the order of selected columns.*

5.2 Sensorgram item settings

Introduction

This section describes the available settings in the ***Settings*** panel at the left of the sensorgram workspace. The ***Select sensorgrams*** panel is described in *Workspace settings, on page 30*.

Data grouping

The ***Data grouping*** setting determines how the data is divided into groups. The number of groups is shown in parentheses in the ***Data grouping*** setting header.

The following ***Data grouping*** settings are available in a ***Sensorgram*** item:

Setting	Description
Flow cell/ Channel	Groups the sensorgrams by flow cell or channel for 1-channel respectively 8-channel data.
Cycle	Groups the sensorgrams by cycle number.
No grouping	Places the sensorgrams from each cycle and flow cell/channel in a separate group.
Custom	Allows free selection of grouping parameters from the list. Parameters may be combined.

Injection assignment

The ***Injection assignment*** setting determines which injections should be used for variable parameter values and response values respectively.

For example, a run may take the sample name from the variable analyte injection but the response for evaluation from an enhancement injection.

Setting	Description
Use variable information from	Variable parameters from the specified injection command will be used as sample parameters in the evaluation.
Use response values from	Response levels from the specified injection command will be used as sample responses in the evaluation.

Alignment

The ***Alignment*** settings determine how the sensorgrams are aligned to zero response and time. The settings are applied to all sensorgrams in the evaluation, and affect only the graphical display in the sensorgram and thumbnail panels.

Choose whether to align to an injection event or a report point. Choose ***No alignment*** to display actual response values. The setting is independent for x- and y-axes.

Sensorgrams are aligned by default to the injection used for response values in ***Injection assignment***, with zero on the x-axis at the beginning of the injection, and zero on the y-axis at the baseline report point.

Select ***Use normalization*** and choose the event or report point to set to a value of 100 to normalize the sensorgrams. Zero is determined by the ***y-axis alignment*** setting. Normalization cannot be used if alignment for the y-axis is turned off.

Sensorgram subtraction

The ***Sensorgram subtraction*** setting supports both blank subtraction and subtraction of a specified cycle. Subtraction is performed within but not between flow cells (1-channel data) or channels (8-channel data).

Subtract blank subtracts the response in blank sensorgrams from all sensorgrams in the same flow cell or channel.

Blank sensorgrams may be sample cycles with solutions defined as ***Blank***, or with zero concentration, or negative control cycles.

Choose the subtraction setting:

Setting	Description
Preceding	Nearest preceding blank. If there is no preceding blank, the nearest blank is used.
Following	Nearest following blank. If there is no following blank, the nearest blank is used.
Nearest	Nearest blank in the cycle sequence.
Median of nearest	Median of the nearest preceding and nearest following blanks.

Subtract cycle subtracts the sensorgrams for a specified cycle from all other others in the same flow cell/channel. Subtraction is not performed if the sensorgrams do not share a common alignment point (for example, solvent correction cycles will not be subtracted from analyte injection cycles).

Color by

The ***Color by*** setting colors sensorgrams according to a selected property. Available options are determined by variable parameters in the run method and by any sensorgram filters that are applied in the evaluation.

Note: *Sensorgram and point colors are hidden by the selection highlight when sensorgrams and points are selected.*

5.3 Selecting data in Sensorgram items

Introduction

This section describes how to select and manage data in ***Sensorgram*** items. Individual sensorgrams can be selected in the sensorgram panel and the table panel. Select sensorgrams to apply curve markers and exclude or include sensorgrams in the evaluation session.

The thumbnail panel supports selection of groups but not individual sensorgrams. Sensorgrams from selected groups are shown in the sensorgram panel.

Selection in the *Thumbnails* panel

Group selection in the thumbnail panel behaves differently according to whether ***Selection as toggle*** is checked in the thumbnail settings (see *Panel toolbar, on page 74*):

Selection as toggle	Description
Unchecked	Click a thumbnail to select the thumbnail. Any previously selected thumbnails will be deselected. Use **Shift-click** and **Ctrl-click** to select multiple thumbnails.
Checked	Click a thumbnail to toggle between selected and deselected. The status of other thumbnails is not affected.

Selected thumbnails are marked with a heavier border.

Selection in the *Sensorgrams* panel

Click on a sensorgram to select or deselect it.

Use ***Select area mode*** (see *General panel toolbar settings, on page 31*) to select multiple sensorgrams in one operation.

Selected sensorgrams are identified with a thicker blue line. The corresponding rows in the table panel are also highlighted.

Click to deselect all selected sensorgrams.

Selection in the *Table* panel

Click on a row to select it. Use **Shift-click** and **Ctrl-click** to select multiple rows.

Selecting a row deselects any previously selected rows unless **Shift-click** or **Ctrl-click** is used.

Selected rows are highlighted in blue. The corresponding sensorgrams are also highlighted.

6 Plot items

About this chapter

Plot items display the data as plots, normally of a response at a selected report point against cycle. Both x- and y-axes can however be customized for other plot requirements.

This chapter describes how to use ***Plot*** items.

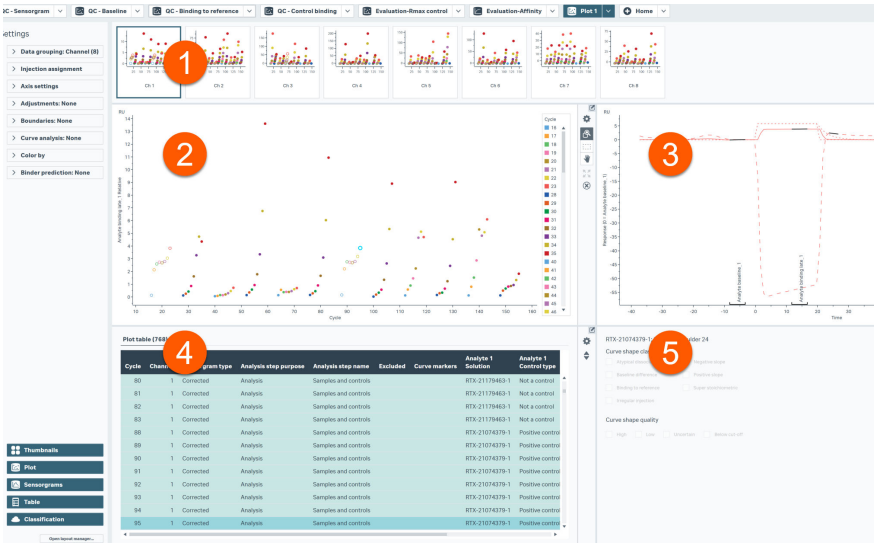
In this chapter

6.1 The Plot workspace

In this section

6.1.1 Introduction

The ***Plot*** item workspace holds up to 5 panels in addition to the ***Settings*** panel at the left of the workspace.



Panel	Content	Description
1	***Thumbnails***	Displays the data groups in the evaluation (see *Section 6.1.2 Thumbnails panel, on page 93*).
2	***Plot***	Displays the data in the selected groups (see *Section 6.1.3 Plot panel, on page 96*).
3	***Sensorgrams***	Displays the sensorgrams for points that are selected in the ***Plot*** panel and table (see *Section 6.1.4 Sensorgrams panel, on page 100*).
4	***Table***	Displays the details for all cycles in the evaluation. Cycles in the selected groups are highlighted (see *Section 6.1.5 Table panel, on page 104*).
5	***Classification***	Displays available classifications and ***Binder prediction*** quality options (see *Section 6.1.6 Classification panel, on page 107*). The panel is visible with the ***Biacore Intelligent Analysis*** extension, when ***Binder prediction*** is active (set to ***Use prediction***).

Drag the panel borders to resize the panels.

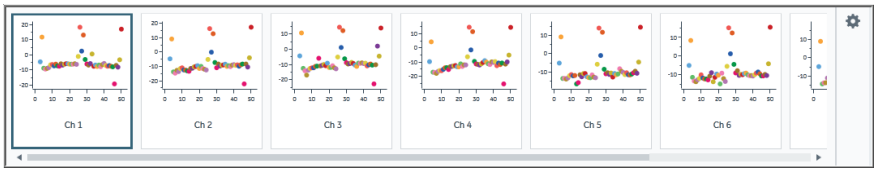
Use the buttons at the bottom of the ***Settings*** panel to show or hide selected panels.

The panels are described in more detail in the following subsections.

6.1.2 Thumbnails panel

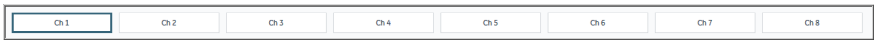
Panel description

The ***Thumbnails*** panel displays thumbnails of the plot data grouped according to the ***Data grouping*** setting. You may need to scroll the panel display to see all thumbnails.



Select one or more thumbnails to display the contents in the ***Plot*** panels. Use **Shift-click** and **Ctrl-click** to select multiple thumbnails. Selected thumbnails are marked by a heavier border.

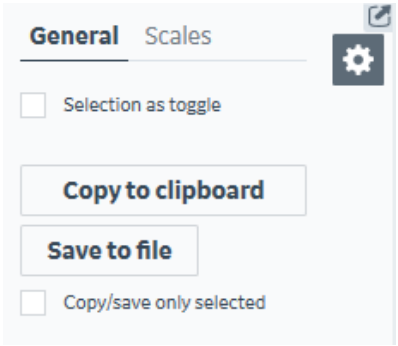
Deselect ***Thumbnails*** in the ***Settings*** panel at the left to collapse the thumbnails to buttons. Data groups can be selected with these buttons even when the thumbnail content is not displayed.



Panel toolbar

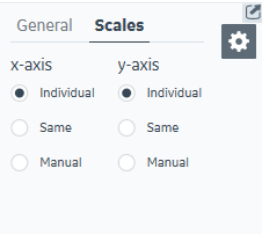
Click ⚙ ***Thumbnail settings*** on the panel toolbar to access display settings for the panel.

General tab



Setting	Description
Selection as toggle	If this option is checked, clicking on a thumbnail will toggle between selected and deselected. When the option is not selected, clicking will only select the thumbnail. ***Note:*** *When the option is checked, using* **Shift-click** *will toggle the status of all thumbnails in the range covered.*
Copy to clipboard	Copies thumbnails to the Windows clipboard as a collection of graphical objects. The thumbnails can be pasted into programs that support pasting for collections of graphical objects. Check ***Copy/save only selected*** to copy only selected thumbnails. If this option is not checked, all thumbnails will be copied.
Save to file	Saves thumbnails as illustrations in ***.png*** (Portable Network Graphics) format. Each thumbnail is saved to a separate file. File names are constructed from the thumbnail label with an added serial number to prevent duplicate names. Check ***Copy/save only selected*** to save only selected thumbnails. If this option is not checked, all thumbnails will be saved.

Scales tab



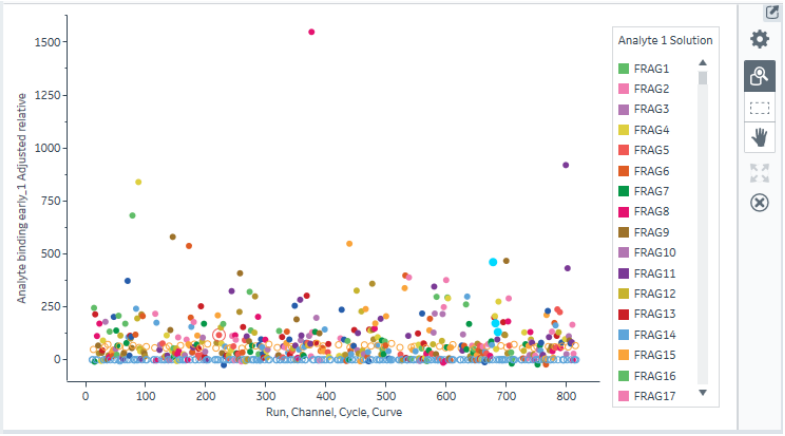
Setting	Description
x-axis ***y-axis***	Determines how the axes will be scaled in the thumbnails. • ***Individual*** scales each thumbnail separately. The scale is determined by the range of values in the thumbnail. • ***Same*** scales all thumbnails to the same scale, determined by the range of values in all thumbnails together. • ***Manual*** scales all thumbnails according to the specified minimum and maximum values.

6.1.3 Plot panel

Panel description

This section describes the plot panel in general plot items. When ***Curve analysis*** is applied, the content of the plot panel is adapted to the analysis (see *Section 6.5.1 Curve analysis plot panel, on page 123*).

The plot panel displays the plot points in the currently selected thumbnail(s).



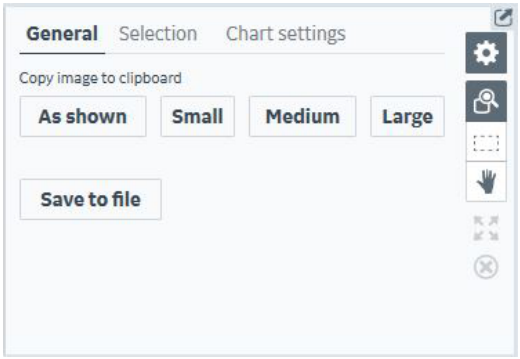
Individual points can be selected in this panel. Click on a point to select/deselect it, or use the ***Area select*** mode to select multiple points by dragging around them (see *General panel toolbar settings, on page 31*). Clicking on successive points adds the points to the selection. Selected points are shown as enlarged blue points, and the sensorgrams from selected points are shown in the sensorgram panel. Use ***Deselect all*** to deselect all selected points.

Panel toolbar

Click ***Chart settings*** on the panel toolbar to access display settings for the panel.

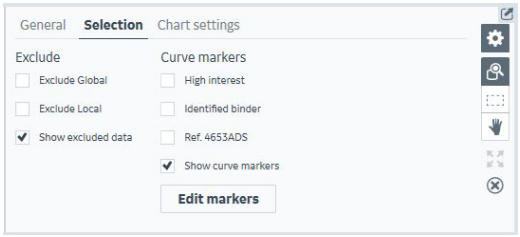
The separate tabs in the chart settings are described below. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.

General tab



Setting	Description
Copy image to clipboard	Copies the plot panel to the Windows clipboard. Choose one of three sizes or ***As shown***. ***Note:*** ***Small***, ***Medium*** *and* ***Large*** *copies use default image proportions.* ***As shown*** *retains the proportions as displayed on the screen.*
Save to file	Saves the plot panel as an illustration in selectable format.

Selection tab

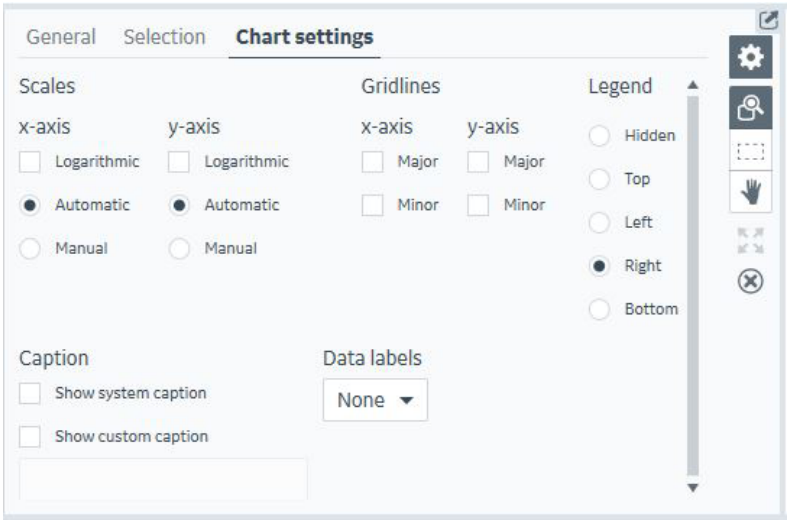


Note: *The curve markers shown here are examples, for illustration purposes only.*

Options on this tab apply to selected plot points. See *Section 6.7 Selecting data in Plot items, on page 146* for details of how to select points.

Setting	Description
Exclude global	Check this option to exclude the selected points and the sensorgrams from which they are derived from all evaluation items, including those already created. If excluded points are selected, the option will be checked. Remove the checkmark to include the points again. Excluding points removes any adjustments and/or table calculations that have been applied.
Exclude local	Check this option to exclude the selected points and the sensorgrams from which they are derived from the current evaluation item only. If excluded points are selected, the option will be checked. Remove the checkmark to include the points again.
Show excluded data	Check this option to show excluded data in the plot panel. Excluded points are shown as gray crosses.
Curve markers	Check the required markers to apply the markers to the selected points. See *Section 3.4 Curve markers, on page 44* for more details.
Show curve markers	Check this option to show curve markers in the plot and thumbnail panels.
Edit markers	Choose this option to manage curve markers. See *Section 3.4 Curve markers, on page 44* for details.

Chart settings tab

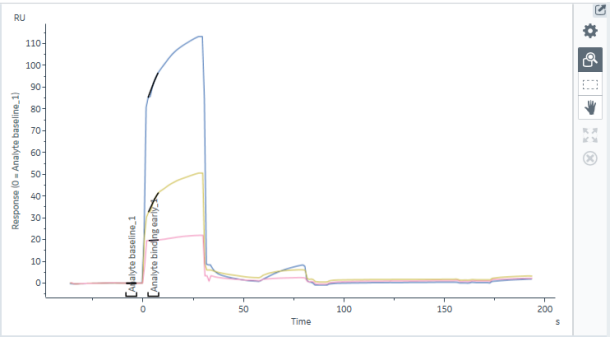


Setting	Description
Scales	Choose ***Logarithmic*** or ***Automatic*** scales for the axes. Automatic scale is always linear for sensorgram data. ***Note:*** *Zero or negative values cannot be displayed on a logarithmic scale.* Choose ***Manual*** and enter minimum and maximum values to set the scales manually.
Gridlines	Choose whether to show major and/or minor gridlines. Gridline spacing is determined according to the scale range.
Legend	Displays a legend identifying points by color. Choose the position of the legend in the panel.
Caption	Displays a caption in the panel. Choose whether to include the system caption and/or a custom caption with text as entered.
Data labels	Displays a label for each point in the plot. Choose the property to use as label text.

6.1.4 Sensorgrams panel

Panel description

The ***Sensorgrams*** panel shows the sensorgrams for points that are selected in the ***Plot*** panel and table. The panel is empty when no points are selected.

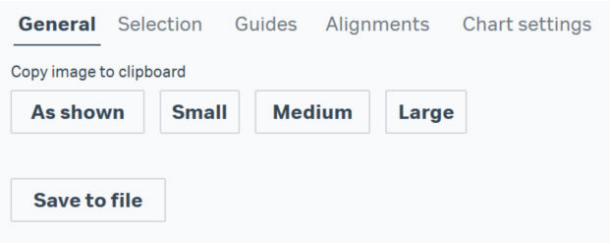


Panel toolbar

Click ***Chart settings*** on the panel toolbar to access display settings for the panel.

The separate tabs in the chart settings are described below. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.

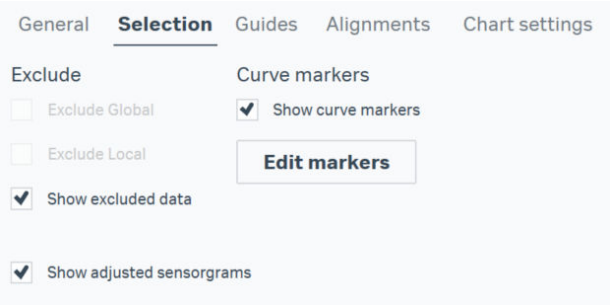
General tab



Setting	Description
Copy image to clipboard	Copies the sensorgram panel to the Windows clipboard. Choose one of three sizes or ***As shown***. ***Note:*** ***Small***, ***Medium*** *and* ***Large*** *copies use default image proportions.* ***As shown*** *retains the proportions as displayed on the screen.*

Setting	Description
Save to file	Saves the sensorgram panel as an illustration in selectable format.

Selection tab



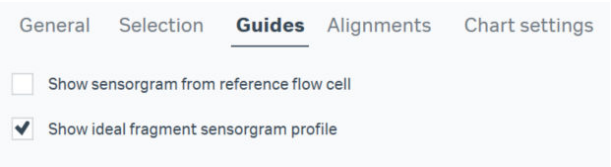
Note: *The curve markers shown here are examples, for illustration purposes only.*

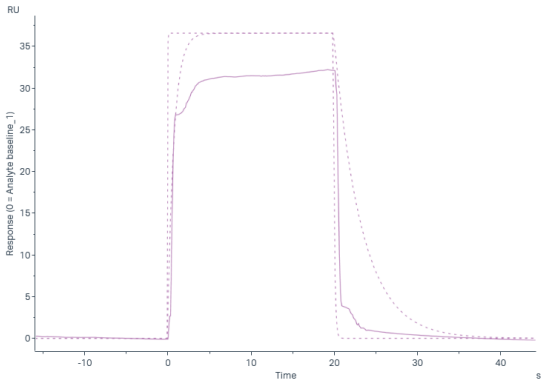
Options on this tab apply to selected sensorgrams. See *Section 5.3 Selecting data in Sensorgram items, on page 88* for details on how to select sensorgrams.

Setting	Description
Exclude global	Check this option to exclude the selected sensorgrams from all evaluation items, including those already created. If excluded sensorgrams are selected, the option is checked. Remove the checkmark to include the sensorgrams again. Excluding sensorgrams removes any sensorgram adjustments and/or table calculations that have been applied.
Exclude local	Check this option to exclude the selected sensorgrams from the current evaluation item only. If excluded sensorgrams are selected, the option is checked. Remove the checkmark to include the sensorgrams again.
Show excluded data	Check this option to show excluded data in the sensorgram panel. Excluded sensorgrams are shown as gray lines.
Curve markers	Check the required markers to apply the markers to the selected sensorgrams. See *Section 3.4 Curve markers, on page 44* for more details.

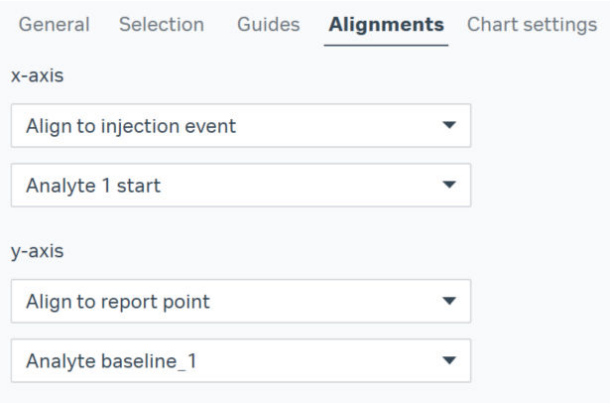
Setting	Description
Solvent corr. out of range	The ***Solvent correction out of range*** marker is automatically set to relevant sensorgrams.
Show curve markers	Check this option to show curve markers in the sensorgram and thumbnail panels.
Edit markers	Choose this option to manage curve markers. See *Section 3.4 Curve markers, on page 44* for details.
Show adjusted sensorgrams	Check this option to view adjusted sensorgrams. The adjustments are set in the ***Settings*** panel to the left of the plot window.

Guides tab



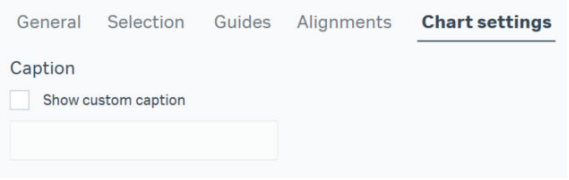
Setting	Description
Show sensorgram from reference flow cell	Check this option to display the sensorgram from the reference cell as a sparsely dotted curve.
Show ideal fragment sensorgram profile	Check this option to display two typical fragment sensorgrams. The sensorgrams appear as dotted curves. One curve shows slight curvature, while the other has a rectangular shape. 

Alignments tab



Setting	Description
x-axis ***y-axis***	Choose how sensorgrams are aligned in the display. The x- and y-axis can be aligned independently to an injection event or a report point. The alignment point will be set to zero in the display.

Chart settings tab



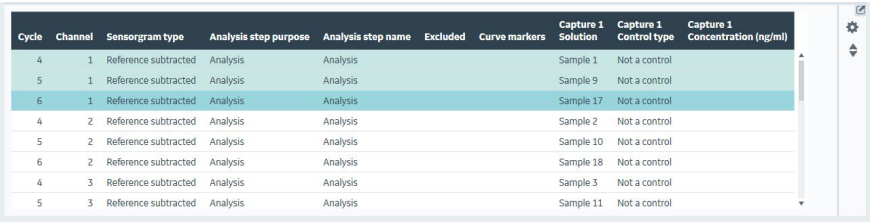
Setting	Description
Caption	Includes a custom caption in the panel. Enter the caption text.

6.1.5 Table panel

Panel description

This section describes the basic functions of the table panel in plot items. When ***Curve analysis*** is applied, the table panel provides additional content (see *Section 6.5.2 Curve analysis table panel, on page 125*).

The table panel lists parameter values for all points included the plot item. Rows for points in the currently selected group are highlighted in light green, and rows for selected points are highlighted in a darker shade.



Cycle	Channel	Sensorgram type	Analysis step purpose	Analysis step name	Excluded	Curve markers	Capture 1 Solution	Capture 1 Control type	Capture 1 Concentration (ng/ml)
4	1	Reference subtracted	Analysis	Analysis			Sample 1	Not a control	
5	1	Reference subtracted	Analysis	Analysis			Sample 9	Not a control	
6	1	Reference subtracted	Analysis	Analysis			Sample 17	Not a control	
4	2	Reference subtracted	Analysis	Analysis			Sample 2	Not a control	
5	2	Reference subtracted	Analysis	Analysis			Sample 10	Not a control	
6	2	Reference subtracted	Analysis	Analysis			Sample 18	Not a control	
4	3	Reference subtracted	Analysis	Analysis			Sample 3	Not a control	
5	3	Reference subtracted	Analysis	Analysis			Sample 11	Not a control	

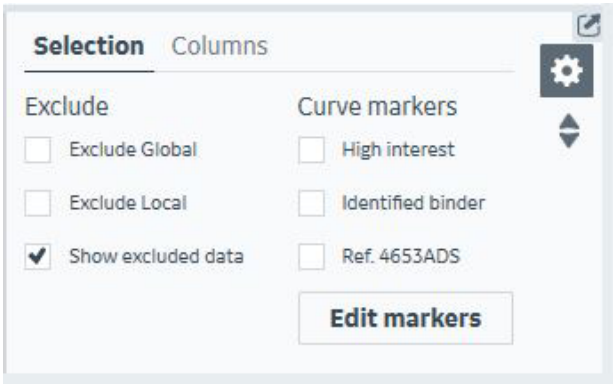
Click on a column header to sort the table by the content of that column.

Click on the funnel shaped filter symbol in the top row to display and select items that can be filtered. When filtering has been performed, the filter symbol turns blue.

Panel toolbar

Setting	Description
Table settings	Opens the display settings for the panel. Tabs in the display settings are described below.
Sort on selection	Groups selected rows at the top or bottom of the table. Click repeatedly to switch between top and bottom of the table.

Selection tab

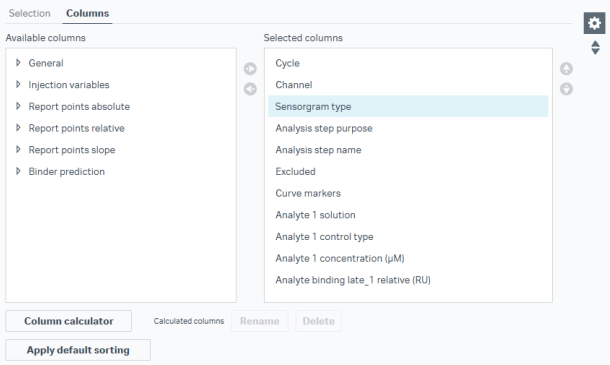


Note: *The curve markers shown here are examples, for illustration purposes only.*

Options on this tab apply to selected table rows. See *Section 6.7 Selecting data in Plot items, on page 146* for details of how to select points.

Setting	Description
Exclude global	Check this option to exclude the selected points and the sensorgrams from which they are derived from all evaluation items, including those already created. If excluded sensorgrams are selected, the option will be checked. Remove the checkmark to include the sensorgrams again.
Exclude local	Check this option to exclude the selected points and the sensorgrams from which they are derived from the current evaluation item only. If excluded sensorgrams are selected, the option will be checked. Remove the checkmark to include the sensorgrams again.
Show excluded data	Check this option to show excluded points and sensorgrams in gray, and to list excluded data in the table. Excluded sensorgrams are identified in the table in the ***Excluded*** column. If this option is not checked, sensorgrams and corresponding table rows are hidden.
Curve markers	Check the required markers to apply the markers to the selected points and sensorgrams. See *Section 3.4 Curve markers, on page 44* for more details.
Edit markers	Choose this option to manage curve markers. See *Section 3.4 Curve markers, on page 44* for details.

Columns tab



Move columns between ***Available columns*** and ***Selected columns*** using the left and right arrow buttons to control the information displayed in the table. Select a column in the ***Selected columns*** list and use the up and down arrow buttons to change the column display order.

Note: *Columns that are not selected cannot be used for axis variables in the plot (see Axis settings, on page 109). If you remove columns from the table that are used as axis settings, the thumbnails and plot will be adjusted.*

Use ***Column calculator*** to add table columns that hold results calculated from other column values. See *Section 6.8 Calculated columns, on page 148* for details.

Click ***Apply default sorting*** to restore the default sort order for table rows if you have changed the sort order.

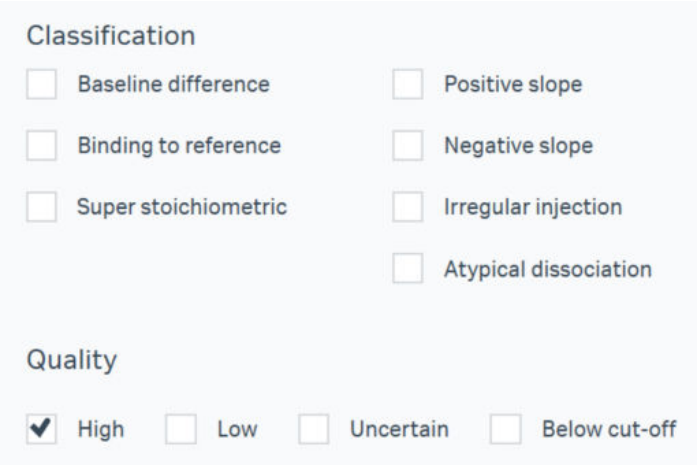
Note: *Applying default sorting does not affect the order of selected columns.*

6.1.6 Classification panel

The ***Classification*** panel is visible when the Biacore Intelligent Analysis extension is active, and when ***Binder prediction*** is active (set to ***Use prediction***).

The ***Classification*** panel is divided into two sections; one for setting ***Binder prediction Classification***, and one for setting ***Binding prediction Quality***.

Classifications and qualities are assigned using check boxes. See *Section 6.6 Biacore Intelligent Analysis for binder prediction, on page 131* for details.



6.2 Plot item settings

Introduction

This section describes the available settings in the ***Settings*** panel at the left of the plot workspace. The ***Select sensorgrams*** panel is described in *Workspace settings, on page 30*.

Data grouping

The ***Data grouping*** setting determines how the data is divided into groups. The number of groups is shown in parentheses in the ***Data grouping*** setting header.

The following ***Data grouping*** settings are available in a ***Plot*** item:

Setting	Description
Flow cell/ Channel	Groups the data by flow cell (1-channel data) or channel (8-channel data).
All together	Plots all the data in a single group.
Channel vs channel	Plots the response in one channel against the same report point in another. Each unique channel pair is treated as a separate group. ***Note:*** *The report points for this kind of plot are selected under* ***Axis settings****.* *This setting can only be used with results from 8-channel instruments.*
Custom	Allows free selection of grouping parameters from the list. Parameters may be combined.

Injection assignment

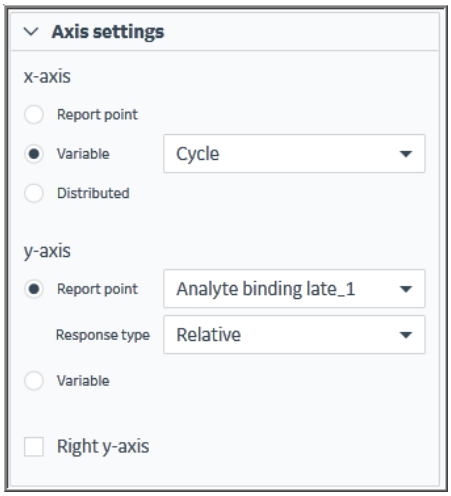
The ***Injection assignment*** setting determines which injections should be used for variable parameter values.

Setting	Description
Use variable information from	Variable parameters from the specified injection command will be used as sample parameters in the evaluation.

Note: *Plot items do not use the* ***Use response values from*** *setting for* ***Injection assignments*** *that is included in other items. Selection of report points for* ***Axis settings*** *provides the corresponding function for plots.*

Axis settings

The ***Axis settings*** determine the parameters used for the x- and y-axes in the plot.



Choose the appropriate axis settings for your plot. For plots that are grouped ***Channel vs channel***, only the report point setting is available.

The ***Distributed*** setting expands the x-axis to avoid plotting multiple points at the same x-axis position.

Note: ***Variable*** *options for axis settings correspond to the columns included in the* ***Table*** *panel. Columns that are not shown in the table panel (see Columns tab, on page 106) cannot be used as axis variables.*

Select ***Right y-axis*** to add a second y-axis to the plot. The settings for the left and right y-axes are independent of each other.

Adjustments

Report point adjustments compensate the response values for blank subtraction, analyte molecular weight, variations in levels of captured ligand and drift in surface binding capacity.

See *Section 6.3 Plot adjustments, on page 111* for details.

Boundaries

Boundaries divide the plot into two or more regions for cut-off or ranking purposes. See *Section 6.4 Cut-off and ranking boundaries, on page 115* for details.

Curve analysis

Curve analysis provides functions for PLA (Parallel Line Analysis) and EC_{50} (half-maximal effective concentration) determinations. See *Section 6.5 Curve analysis (PLA and EC50), on page 121* for details.

Color by

The ***Color by*** setting colors the plot points according to a selected property. Available options are determined by variable parameters in the run method and by any curve filters that are applied in the evaluation.

Note: *Sensorgram and point colors are hidden by the selection highlight when sensorgrams and points are selected.*

Binder prediction

Binder prediction is only available when the ***Biacore Intelligent Analysis*** extension is active and when a boundary that is not ***None*** or ***Ranking*** has been set. It is designed for predicting the quality of results in a binding level screen run.

Select ***Use prediction*** to activate the prediction content. Select a prediction model and model version and click ***Predict*** to predict the quality of the results.

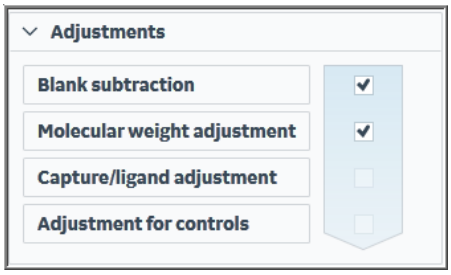
Click ***Train and save new version*** when changes have been made in the ***Classification*** panel during the review process to gradually change the prediction model to your preferences.

Training and saving new versions can only be performed on the latest available version of the prediction model.

6.3 Plot adjustments

General features

Adjustments compensate the plot points for background response (blank subtraction), analyte molecular weight, variations in levels of captured ligand and drift in surface binding capacity.



To apply an adjustment using the most recent settings (or default settings if none have previously been used), select the checkbox. Click the appropriate button to view or change the settings. Adjustments that have been applied are indicated by a check-mark. Remove the checkmark to remove an adjustment.

Adjustments should be applied in the order listed from top to bottom, since adjustments later in the sequence can be affected by earlier ones. Applying an adjustment will cancel any adjustments later in the sequence that have already been applied.

Adjustments are synchronized between the plot and sensorgram panels.

Adjustment preview

The workspace for ***Blank subtraction*** and ***Adjustment for controls*** allows selection of appropriate parameters and provides a preview of the adjustment effect. The workspace for ***Blank subtraction*** is illustrated below.

- Select the report point to show in the before-after preview under ***View adjustments for***. You can preview the adjustment effect on any report point, regardless of which is selected in the plot item.
- The table lists one row for each flow cell (1-channel data) or channel (8-channel data). Select a row to preview the adjustment effect for the selected data.
- Adjustment settings are independent for each flow cell (1-channel data) or channel (8-channel data). Use the setting in the table header to apply the same setting to all flow cells/channels: change settings for individual flow cells/channels in the table row.
- Select ***Apply and close*** to apply the adjustment.

Note: *Points may be excluded (locally or globally) in the preview plots. The exclusion will apply even if the adjustment is not applied.*

Blank subtraction

Blank subtraction subtracts the response in a blank sensorgram from all sensorgrams (within the same flow cell or channel in data from 1-channel respectively 8-channel instruments). For evaluation sessions that include multiple runs, blank subtraction is performed separately within each run.

Blanks may be sample cycles defined as ***Blank*** or ***NegativeControl***, or sample cycles with zero concentration.

Choose the subtraction setting:

Setting	Description
Nearest	Nearest blank in the cycle sequence.
Average nearest	Average of the nearest preceding and nearest following blanks.
Preceding	Nearest preceding blank. If there is no preceding blank, the nearest following blank is used.
Following	Nearest following blank. If there is no following blank, the nearest preceding blank is used.

Molecular weight adjustment

Molecular weight adjustment adjusts the response by dividing it by the analyte molecular weight, so that responses are visualized on a molar basis instead of weight basis. The response is expressed as ***RU/100 Da*** (response per 100 daltons).

Any samples for which no molecular weight is available or for which the molecular weight is set to zero will be excluded from the adjusted plot.

Capture/ligand adjustment

Capture/ligand adjustment compensates for cycle-to-cycle variations in levels of captured ligand by dividing the analyte response by the level of captured ligand. Adjusted responses are expressed as sample response divided by capture level.

Adjustment for controls

Adjustment for controls compensates for drift in the analyte binding capacity by normalizing response levels relative to negative and positive control responses from control samples repeated at intervals throughout the run. A positive control is required. The negative control level is set to zero if no negative control is specified. The normalized responses are plotted on a scale of 0% to 100%.

For evaluation sessions that include multiple result sets, adjustment for controls is applied separately to each result set. The adjustment also has the effect of normalizing the response levels between result sets provided that the same control compound is used in all result sets.

The adjustment is performed as follows:

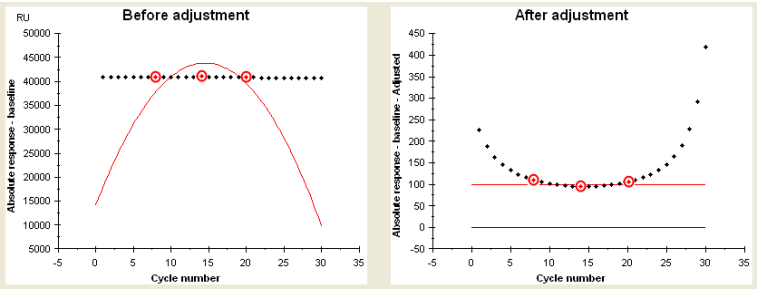
1. Curves are fitted to the control sample responses for positive and (if used) negative controls, using either ***Linear*** or ***Polynomial*** fitting equations.

Fitting	Equation
Linear	$y = ax + b$ (where ***a*** and ***b*** are constants)
Polynomial	$y = ax^2 + bx + c$ (where ***a***, ***b*** and ***c*** are constants)

2. The fitted line(s) are transformed to straight horizontal lines with values 100 for the positive control and 0 for the negative control.
3. The corresponding transformation is applied to all points in the plot (including the actual control sample responses), to normalize the points relative to the fitted control responses.

Adjustment for controls cannot be applied in regions where the positive control curve lies below the negative control level. Any points that lie in such regions will be excluded from the adjusted plot.

Note: *Beware of using a polynomial fitting function with less than 4 control samples. The parabolic curve created by the function can deviate greatly from the points, leading to adjustment that does not reflect the drift in the control responses. An extreme example is illustrated below.*



6.4 Cut-off and ranking boundaries

Introduction

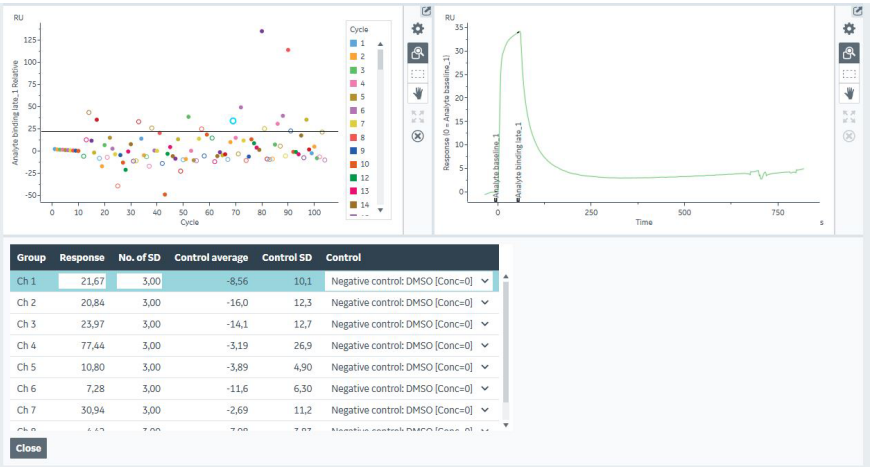
Boundaries divide the plot into two or more regions for cut-off or ranking purposes. Different boundary types can be applied, according to the needs of the evaluation. The boundary type is applied to all groups in the evaluation, although settings may differ between groups. Once boundaries have been set, their position can be adjusted either by editing the settings for the boundary or by dragging the boundary line in the plot panel.

Boundary type	Description
Control based cut-off	Creates a single boundary based on the response from a selected control sample.
Binding level screen cut-off	Creates a single boundary based on the fraction of points that lie above the threshold, with consideration for deviant binding behavior. This option is primarily intended for fragment screening applications, and is only available when the ***Extended Screening*** or the ***Biacore Intelligent Analysis*** extension is active.
Manual cut-off	Creates a single boundary at a specified response level.
Ranking	Creates multiple boundaries that divide the data into groups (e.g., no binding, weak binding and strong binding).

Cut-off and ranking plots are opened in edit mode when first created, allowing the boundary properties to be defined. Click ***Close*** in the edit panel to restore the table display. Click ***Edit*** in the ***Settings*** panel on the left to re-open edit mode.

Control based cut-off

Control based cut-off creates a single cut-off boundary with default settings at a response calculated as the average response for a selected control sample plus 3 standard deviations (SD).



The following parameters can be set for ***Control based cut-off***:

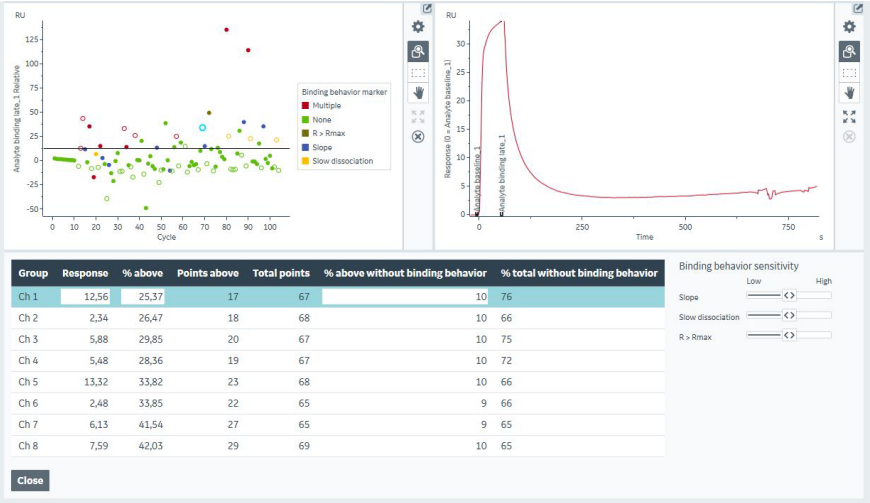
Parameter	Description
Response	The actual response level of the cut-off. If you change this value, the ***No. of SD*** value is recalculated accordingly.
No. of SD	The number of standard deviations added to the average control response. If you change this value, the actual response level for the cut-off is recalculated accordingly.
Control	Select the sample to use as a control.

A ***Cut-off*** column is added to the table, with rows marked as either ***Above*** or ***Below***.

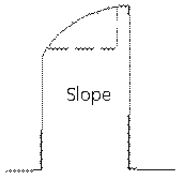
Binding level screen cut-off

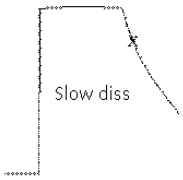
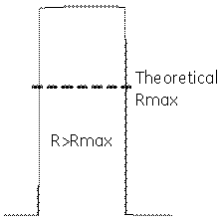
Binding level screen cut-off is primarily intended for evaluation of fragment binding assays, where the focus of the experiment is often to reduce the number of fragments taken for continued study, rather than to identify unequivocal binders. The function creates a single cut-off boundary based on the number of samples (excluding control samples) above the cut-off (considered as "hits").

Points in a ***Binding level screen cut-off*** plot are colored by default by binding behavior marker.



The function also analyses the sensorgrams to identify atypical binding behavior that is relatively common in fragment screening work. The plot points are colored by binding behavior indicators when ***Binding level screen cut-off*** is applied. The following atypical binding may be identified:

Name	Description	Sensorgram appearance
Slope	Indicates a significantly increasing response during sample injection instead of the normally expected rapid binding to a steady-state level. ***Slope*** is determined from the average slope of the sensorgram between the report points ***binding early*** (shortly after injection start) and ***binding late*** (shortly before injection end).	

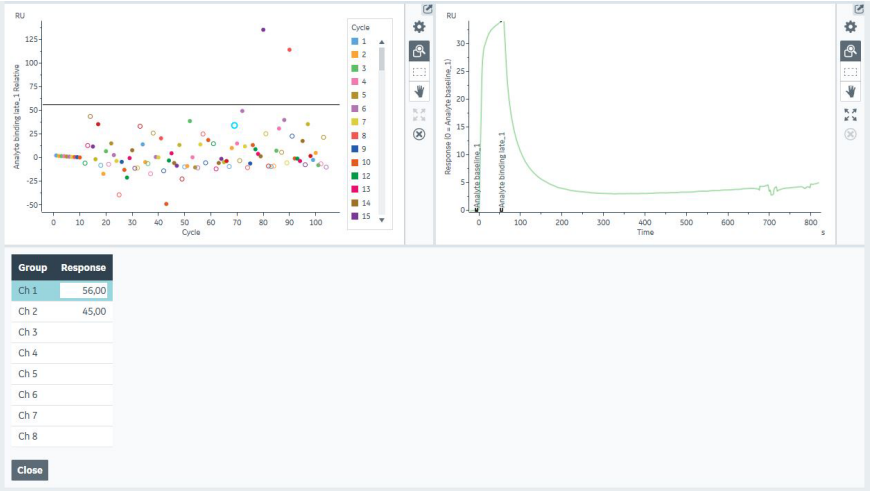
Name	Description	Sensorgram appearance
Slow diss	Indicates that the compound does not dissociate immediately after the end of the sample injection. ***Slow diss*** is determined from the relative response above baseline for the report point ***stability early*** (shortly after the end of the injection).	
R>Rmax	Indicates that the maximum response reached during sample injection is higher than expected for the ligand immobilization level. ***R>Rmax*** is determined from the highest response during sample injection and the theoretical maximum binding capacity based on 1:1 binding. Calculation of the theoretical maximum binding capacity requires the molecular weight of the ligand, provided either during immobilization or edited in the ***Chip Information***. This parameter is not supported for assays that use captured ligands.	

The following parameters can be set for ***Binding level screen cut-off***:

Parameter	Description
Response	The actual response level of the cut-off.
% above	Percentage of the total number of samples (excluding control samples and samples that have been excluded from the plot) that lie above the cut-off.
% total without binding behavior	Percentage of the total number of samples (excluding control samples and samples that have been excluded from the plot) that lie above the cut-off and have no binding behavior indicator.
Binding behavior sensitivity	Move the sliders to adjust the sensitivity of binding behavior detection. Move to the left to reduce the sensitivity (detect fewer points) and to the right to increase sensitivity (detect more points).

Manual cut-off

Manual cut-off creates a single boundary at a specified response level. Enter the level in the boundary settings table. Different levels may be specified for different groups.



Ranking

Ranking boundaries divide the plot into a user-defined number of regions. Ranking boundaries are set automatically on the basis of average responses for control samples and may be edited freely. Custom boundaries can also be added.

Ranking boundaries are identified by name and a numerical value. The numerical value is intended to aid data processing in third-party software.

Note: *The ranking value does not affect the response level of the boundary.*

Points are ranked in the plot table as ***Above ...*** the name of the nearest lower boundary. Points below the lowest boundary are ranked as ***Below*** that boundary name. Boundaries are also assigned a numerical value that can be useful in data processing in third-party software. Points below the lowest boundary are assigned a ranking value of zero.

Follow the steps below to manage ranking boundaries.

Step	Action
1	Choose ***Ranking*** from the ***Boundaries*** options.

2	To add a new boundary, click ***Edit rankings***, then choose ***Add boundary*** in the dialog box. Enter the name and ranking value for the new boundary. ***Note:*** *Ranking values are required but do not have to be unique.* Click ***Apply and close*** when you have added all the boundaries you need.
3	Enter boundary levels for the new boundaries.
4	To edit a boundary level, enter a new level in the table or drag the boundary line in the plot.
5	To edit a boundary name or ranking value, click ***Edit rankings*** and change the settings.
6	To delete a boundary, click ***Edit rankings***, then select the boundary and choose ***Remove boundary***.

6.5 Curve analysis (PLA and EC_{50})

In this section

Introduction

The ***Curve analysis*** plot setting provides functions for evaluation of relative potency in comparison with a reference substance using either PLA or EC_{50}. Both functions are based on a plot of response against the logarithm of concentration. Equations for the fitting models are given in *Appendix A.3 Other fitting models, on page 296*.

- PLA estimates relative potency by fitting the linear region of the sample and reference plots to a model with common slope and individual intercepts. The relative potency is determined from the difference in intercept values.
- EC_{50} determines the effective concentration for half-maximal response from the plot. Relative potency is determined by comparing the EC_{50} values for test and reference substances.

Relative potency is determined separately for each test substance relative to each reference substance within the same data group.

Applying ***Curve analysis*** changes the information presented in the ***Plot*** panel and the ***Table*** panel.

Curve analysis is only available if the ***Concentration & Potency*** extension is active.

Note: *Applying **Curve analysis** will remove any boundaries that have been set in the plot and vice versa.*

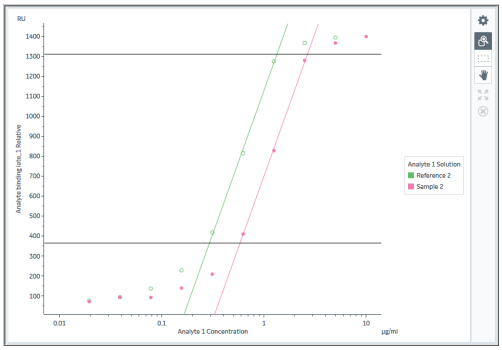
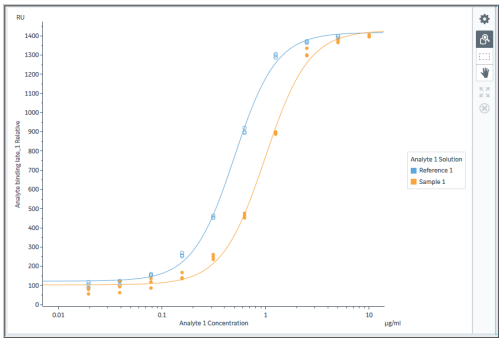
Requirements

Requirements for PLA and EC_{50} determination are summarized in the table below.

Measure-ment	Requirements
PLA	• A concentration series of one or more reference substances, covering the linear region of a plot of response against the logarithm of the concentration. • A concentration series of one or more test substances, covering the linear region of a plot of response against the logarithm of the concentration. The concentrations of reference and test substances do not have to be the same.
EC_{50}	A concentration series of each test substances, sufficiently wide to cover the approach to asymptotes at the lowest and highest concentrations. A concentration series of one or more reference substances is required for calculation of relative potency.

6.5.1 Curve analysis plot panel

Panel description

Curve analysis	Plot panel
PLA	The plot panel for PLA shows a plot of binding response against analyte concentration, with a logarithmic concentration axis. Points that lie between adjustable horizontal boundaries are fitted to a straight line with common slope and individual intercepts for each pair of test and control substances within the group. Drag the boundary lines to adjust the region used for fitting. Fitting modes and parameters can be changed from the table panel. 
EC50	The plot panel for EC_{50} shows a plot of binding response against analyte concentration, with a logarithmic concentration axis. Points for each analyte concentration series are fitted to a 4-parameter equation. Fitting modes can be set from the table panel. 

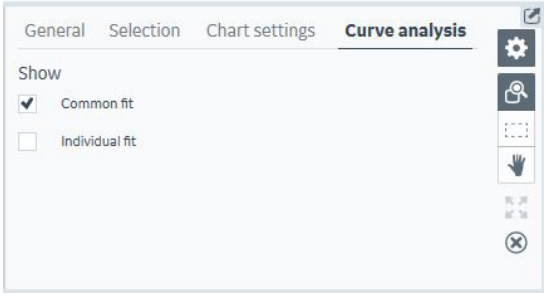
Panel toolbar

Click ***Chart settings*** on the panel toolbar to access display settings for the panel.

The separate tabs in the chart settings are described below. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.

Chart settings

For PLA, the settings tab ***Curve analysis*** is added to the chart settings (see *Section 6.1.3 Plot panel, on page 96* for a description of other tabs in chart settings).



Data is automatically fitted using both ***Common fit*** and ***Individual fit***. The setting here affects the way the data is displayed in the PLA plot panel as follows:

Setting	Description
Common fit	Displays the fit to both reference and sample with a common slope value.
Individual fit	Displays the fit to each concentration series separately.

If both options are selected, an overlay plot of the two modes will be displayed.

6.5.2 Curve analysis table panel

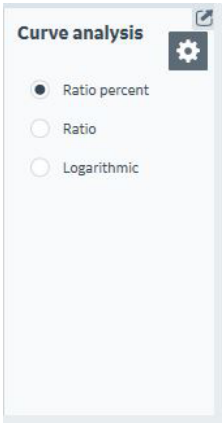
General panel description

When ***Curve analysis*** is applied, the table panel presents three tabs. The illustration below shows the panel for PLA:

Tab	Description
Plot table	Provides the same functionality as the table panel in general plot items (see *Section 6.1.5 Table panel, on page 104*), with the addition of the ***Curve analysis*** option in ***Table settings*** (see *Chart settings, on page 124*).
Results	Displays the numerical results of the evaluation.
Settings/Settings and parameters	Provides ***Fit mode*** options and displays settings and parameters for the numerical values (see *PLA settings, on page 127* and *EC50 settings and parameters, on page 129*).

Panel toolbar

Click ***Table settings*** on the panel toolbar to access ***Curve analysis*** settings.



Setting	Description
Ratio percent	Relative potency is expressed as the sample potency as a percentage of the reference potency.
Ratio	Relative potency is expressed as the ratio of the sample potency to the reference potency.
Logarithmic	Relative potency is expressed as the logarithm (base 10) of the ratio of the sample potency to the reference potency.

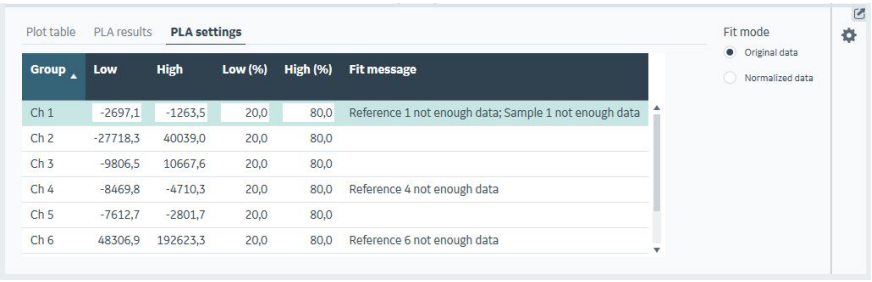
PLA results

The ***PLA results*** tab in the table panel shows the numerical results of the PLA determination.

Parameter	Description
Group, ***Control***, ***Sample***	Identification of the sample-control pair.
Relative potency	Relative potency of the sample compared to the control.
95% confidence	Lower and upper limits of the confidence interval for the relative potency calculation.
Slope	Slope of the fitted straight line. This parameter is reported for the following cases: • Common (obtained when sample and control lines are constrained to the same slope) • Sample • Control
R2	Regression coefficient for the straight line fitting. This parameter is reported for the following cases: • Common (obtained when sample and control lines are constrained to the same slope) • Sample • Control
Slope ratio S/C	Ratio of the sample slope and the control slope.

PLA settings

The ***PLA settings*** tab in the table panel provides ***Fit mode*** options and lists the settings for the fitting boundaries.



Group	Low	High	Low (%)	High (%)	Fit message
Ch 1	-2697,1	-1263,5	20,0	80,0	Reference 1 not enough data; Sample 1 not enough data
Ch 2	-27718,3	40039,0	20,0	80,0	
Ch 3	-9806,5	10667,6	20,0	80,0	
Ch 4	-8469,8	-4710,3	20,0	80,0	Reference 4 not enough data
Ch 5	-7612,7	-2801,7	20,0	80,0	
Ch 6	48306,9	192623,3	20,0	80,0	Reference 6 not enough data

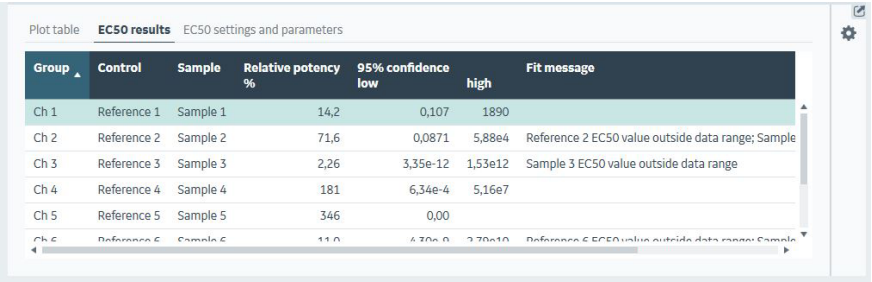
The boundaries (***Low*** and ***High***) are listed as both response values and as a percentage of the range between the asymptotes. Boundaries can be adjusted either by dragging in the plot panel or by entering new values in the settings tab.

Fit mode provides the following options:

Mode	Description
Original data	Fits to the original response values.
Normalized data	Fits to data normalized separately for each curve to the lower and higher asymptote values.

EC50 results

The ***EC50 results*** tab in the table panel shows the numerical results of the EC50 determination.



Group	Control	Sample	Relative potency %	95% confidence low	high	Fit message
Ch 1	Reference 1	Sample 1	14,2	0,107	1890	
Ch 2	Reference 2	Sample 2	71,6	0,0871	5,88e4	Reference 2 EC50 value outside data range; Sample
Ch 3	Reference 3	Sample 3	2,26	3,35e-12	1,53e12	Sample 3 EC50 value outside data range
Ch 4	Reference 4	Sample 4	181	6,34e-4	5,16e7	
Ch 5	Reference 5	Sample 5	346	0,00		

Reported values in addition to sample identification are listed below.

Parameter	Description
Relative potency	Relative potency of the sample compared to the control.
95% confidence	Lower and upper limits of the confidence interval for relative potency calculation.

EC50 settings and parameters

The ***EC50 settings and parameters*** tab in the table panel provides ***Fit mode*** options and lists the parameters for the 4-parameter fitting.

Reported parameters are listed below. See *4-parameter and EC50, on page 296* for details of the 4-parameter fitting function.

Parameter	Description
Group, ***Curve***	Sample identification.
EC50	EC_{50} value (concentration for half maximal response).
Lower asymptote	Lower asymptote value for the fitted curve.
Upper asymptote	Upper asymptote value for the fitted curve.
Hill coeff	Slope of the fitted curve at the EC_{50} value.
Chi²	Chi-square statistical fitting parameter.
Function	Fitting function. Only 4-parameter fitting is currently supported.

Fit mode provides the following options:

Mode	Description
Original data	Fits to the original response values.
Normalized data	Fits to data normalized separately for each curve to the lower and higher asymptote values.

Mode	Description
Restricted fit	Fitting is constrained so that each sample-control pair have the same upper and lower asymptotes and Hill coefficient. This aids evaluation of data where the range of sample concentrations is not sufficient for reliable fitting on its own. ***Note:*** *If the concentration range of both sample and control series is insufficient for reliable fitting, the reported results should be discarded.*

6.6 Biacore Intelligent Analysis for binder prediction

In this section

6.6.1 Introduction

Binding level screen is an assay to identify potentially interesting compounds based on level of binding to the target and overall sensorgram shape. The ***Biacore Intelligent Analysis*** extension can automate the evaluation of such an assay by using a machine learning algorithm to classify each interaction. This can save time, provides confidence during evaluation, and reduces the risk of selecting compounds with intrinsic problems such as non-specific binding and aggregation.

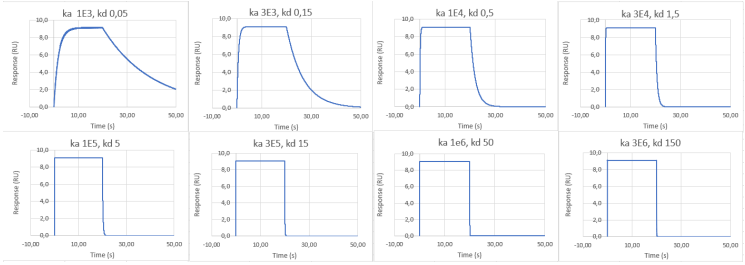
The ***Biacore Intelligent Analysis*** extension comes with a predefined evaluation method and a pretrained prediction model for fragment binding level screen. If you do not completely agree with the outcome of the prediction model, you can train it with your changes or start with an empty model. This makes it better adapted to your data, needs and interpretations in future evaluations.

For binding level screens, Biacore Intelligent Analysis compares properties of the measured data with properties of the data used for training to predict if it is of high or low quality.

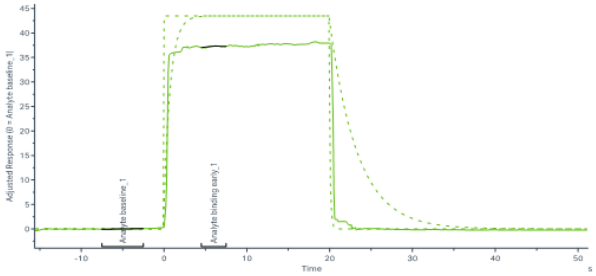
Biacore Intelligent Analysis for binding level screen evaluations is referred to as ***Binder prediction*** in the software.

Examples of ideal and non-ideal fragment profiles

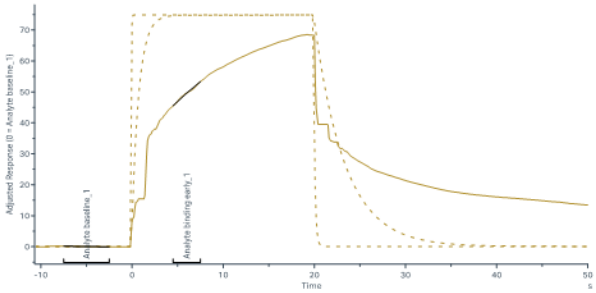
Fragment affinities to their targets are generally in the µM to mM range. The images below show simulated binding profiles of fragments with a 50 µM affinity at a concentration of 500 µM. Slight curvature can be observed for the fragments with low k_a and k_d, but in most cases the profiles are characterized by a rapid approach to steady state and instant return to the baseline during dissociation.



The image below shows a ***High*** quality fragment sensorgram (solid line) in overlay with ideal fragment sensorgram profiles (dotted lines). It is only impacted by small disturbances at the injection start and stop, and rapidly reaches steady state and dissociates rapidly.

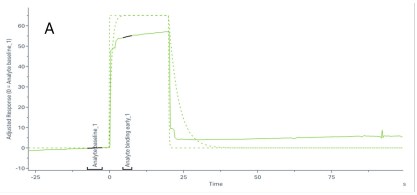


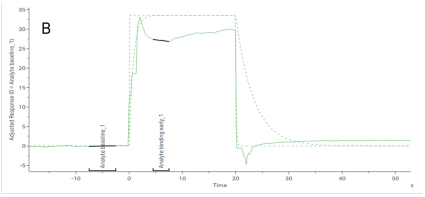
Deviations from the ideal fragment sensorgram profile can have many reasons, such as secondary binding events, solubility issues, aggregation, matrix effects, binding to the reference surface, memory effects, or poor blank subtraction. The image below shows a ***Low*** quality fragment sensorgram (solid line) in overlay with ideal fragment sensorgram profiles (dotted lines). Steady state has not been reached at the report point set six seconds after injection start and the signal remains high even after 30 seconds of dissociation.

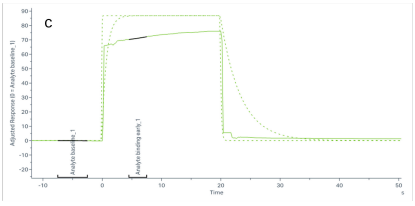


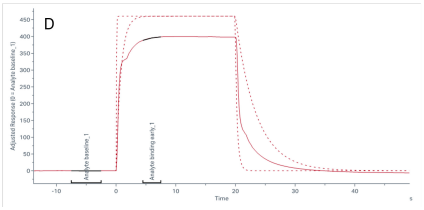
The prediction model always provides at least one classification for fragment results that are assigned ***Low*** quality, but that does not mean that all compounds receiving classifications are predicted to be of ***Low*** quality. In the image below, compounds have an ***Atypical dissociation*** (A), ***Irregular injection*** (B), and ***Positive slope*** (C), but the deviations are considered small and the fragments are assigned ***High*** quality.

In contrast, compound D has an ideal profile but the binding level is much higher than the positive control (not included in the image below). This results in the classification ***Super stochiometric*** which always causes a ***Low*** quality. For more information about the classifications, see *Classification panel, on page 141*.


A
Time
s


B
Time
s


c
Time
s


D
Time
s

6.6.2 Recommendations for binding level screen runs

Fragment based screens typically require solvent correction. Additionally, the following components are required for the predefined Biacore Intelligent Analysis evaluation method for fragment binding level screen:

- **Negative controls (blanks)** are used for blank subtraction.
- **Positive controls** are used to correct data for trends in ligand activity. The positive control should be injected at a high enough concentration to reflect the maximum binding activity. This is important since the positive control is additionally used to determine the binding level limit for super stoichiometric binders.

  If no positive controls are available, the immobilization level and molecular weight of the ligand together with the molecular weight of samples, are used for determination of super stoichiometric binders.

  Note: *The calculations will not be correct if the ligand is captured. Runs with captured ligands require a positive control.*
- **The molecular weight** of samples and positive controls are used for molecular weight adjustment of binding levels.

Tip: *The predefined run method for fragment binding level screen includes all required components.*

The available pretrained Biacore prediction model for fragment binding level screen has been trained with blank subtraction, molecular weight adjustment, and adjustments for controls. Because of this, sample data adjusted differently may not be predicted as accurately with the pretrained Biacore model.

6.6.3 Workflow for binding level screen evaluation using Biacore Intelligent Analysis evaluation method

This section describes the recommended way of evaluating affinity screen data with Biacore Intelligent Analysis: with a predefined evaluation method.

Follow the steps below to evaluate using an evaluation method for Biacore Intelligent Analysis.

Step	Action
1	Select one or several binding level screen runs in Biacore Insight Evaluation Software.
2	Apply a Biacore Intelligent Analysis evaluation method for fragment binding level screen.
3	Apply the solvent correction. *Result:* • Data is opened and adjusted according to settings, typically reference subtraction and correction for molecular weight and ligand activity decline. • Binding levels of adjusted samples are presented. Samples below the boundary are set to ***Below cut-off***. • The quality of the sample sensorgrams receive classifications and are presented as being of ***High***, ***Low*** or ***Uncertain*** quality. • The table is sorted by ***High quality Certainty (%)*** to bring the high quality compounds to the top.
4	Confirm that the controls are acceptable, found in the ***QC - Control binding*** item. Globally exclude any controls with deviating curve shape or amplitude from the right-click menu of the point or sensorgram. If any controls are excluded, perform another prediction from the ***Binder prediction*** setting (see *Binder prediction, on page 110*).
5	(Optional) Clone the ***Evaluation – Binding level*** item before proceeding to step 6. This makes it possible to later compare the originally predicted result with the final results.
6	Review the predicted results, in particular any ***Uncertain*** results. If you do not agree with the predictions, change them in the ***Classification*** panel. See *Section 6.6.5 Review the results, on page 139* for more information.
7	(Recommended) If a significant number of changes were made in step 6, train a new version of the prediction model from the ***Binder prediction*** setting. This adapts the model better to your data and definition of quality. See *Train and save new version, on page 144*.

Step	Action
8	Find the compounds of interest, for example by filtering the plot table on ***Binder Quality: High*** and sorting the report point column ***Analyte binding early_1 Adjusted relative*** from high to low values. See *Table panel, on page 140* for more information.
9	Save the evaluation. ***Note:*** *In the predefined evaluation methods, the prediction model is set to version 1 in* ***Binder prediction****. To achieve a continuous training of models, it is recommended to change the version to* ***Latest*** *when creating a new evaluation method.*

6.6.4 Workflow for binding level screen evaluation using manual application of Biacore Intelligent Analysis

This section describes the second way of evaluating affinity screen data with Biacore Intelligent Analysis: To apply the prediction manually.

Follow the steps below to manually apply a prediction to already opened data.

Step	Action
1	Confirm that the run contains all necessary controls and that required information is available in the ***Variables*** workspace. See *Section 6.6.2 Recommendations for binding level screen runs, on page 135*.
2	Globally exclude any positive or negative controls with deviating curve shape or amplitude from the right-click menu of the point or sensorgram. Depending on evaluation method, controls can be found in the ***QC - Control*** binding item. If not, create a new ***Plot*** item and display only the controls.
3	Create a new ***Plot*** item in the ***Home*** workspace.
4	Plot the adjusted response of ***Analyte binding early_ 1***. Recommended adjustments are blank subtraction, molecular weight adjustment and adjustment for controls. See *Section 6.3 Plot adjustments, on page 111* for more information.
5	Set a boundary in ***Settings***. ***Note:*** *The boundary* ***Ranking*** *is not compatible with Biacore Intelligent Analysis.* See *Section 6.4 Cut-off and ranking boundaries, on page 115* for more information.
6	Select a prediction model and perform the prediction from ***Binder prediction*** in ***Settings***. See *Binder prediction, on page 110* for more information.
7	Perform steps 4-8 described in *Section 6.6.3 Workflow for binding level screen evaluation using Biacore Intelligent Analysis evaluation method, on page 136*.

6.6.5 Review the results

It is recommended to review the results of the prediction, to ensure a high quality of the evaluation. In particular:

- Go through the ***Uncertain*** results and set ***Low*** or ***High*** quality. It is possible to display only these results in the table. When you have a model that you trust, these results may be the only ones needing attention.
- Edit the predicted quality or classifications if you do not agree with any of the predictions.

Changes to predictions are done in the ***Classification*** panel and apply to selected data. See the following information about each panel for details about the review process.

Note: *All manual changes are discarded if you perform another prediction.*

Plot panel

By using the predefined evaluation method, ***Blank subtraction***, ***Molecular weight adjustment*** and ***Adjustments for controls*** are all performed in the ***Evaluation-Binding level*** item. The y-axis in the plot has the unit % of positive control. In the plot, samples are colored by Binder prediction quality. The quality N/A is used for positive controls. The boundary line is visible. This view is focused on flow cells for 1-channel data and channels in 8-channel data (x-axis plot order ***Run, Flow cell/Channel, Cycle, Curve***) and it is easy to follow trends over time in each flow cell or channel.

The x-axis order ***Run, Cycle, Flow cell/Channel, Curve***, changeable from ***Axis settings***, can instead be useful if compounds from the same structure classes are run in consecutive cycles.

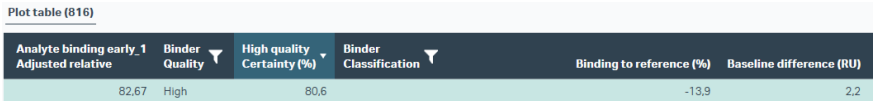
Sensorgrams panel

Sample sensorgrams are adjusted in the same way as the plot data. In the ***Guides*** tab, found in the ***Sensorgrams*** panel settings, it is possible to activate guiding sensorgrams. Ideal fragment sensorgram profiles (see *Examples of ideal and non-ideal fragment profiles, on page 132*) are scaled in relation to the sample(s) and presented as dashed lines in overlay plot with sample sensorgram(s). The sensorgram from the reference flow cell is not adjusted and is best viewed in the context where ***Show adjusted sensorgrams*** is unchecked in the ***Selection*** tab.

For general information about the sensorgrams panel, see *Section 6.1.4 Sensorgrams panel, on page 100*.

Table panel

The following image shows the result table with columns that are specific for ***Binder prediction***.



Column	Description
Plot table (xxxx)	The line above the table displays the number of samples used in the evaluation. The number is updated when a filter is applied.
Analyte binding early_1 Adjusted relative	This column is derived from the ***Binding Early*** report point with adjustments applied, and reflects the relative binding activity of a compound. With molecular weight adjustments, the response is related to binding stoichiometry, and with adjustments for controls the binding activity is expressed in percent of the positive control.

Column	Description
Binder Quality	The predicted quality of the binder result. It is related to the ***High quality Certainty***: • ***Low*** corresponds to a ***Certainty*** close to 0%. • ***Uncertain*** corresponds to a ***Certainty*** of approximately 50%. • ***High***, without detrimental classifications, corresponds to a ***Certainty*** of 100%. ***Below cut-off*** corresponds to a binding curve with a response below the boundary level.
High quality Certainty (%)	The predicted certainty that the results are of high quality, scaled between 0% to 100%.
Binder Classification	The predicted classifications of the binder. For more information, see *Classification panel, on page 141*.
Binding to reference (%)	A high value can indicate that the remaining binding to the reference surface after the analyte injection is significant.
Baseline difference (RU)	The baseline difference between the following cycle and present cycle. A high value can indicate that the sample blocks the target, which can impact binding in subsequent cycles.

Filters can be applied to columns ***Binder Quality*** and ***Binder Classification***. The filter funnel turns blue when a filtration is active.

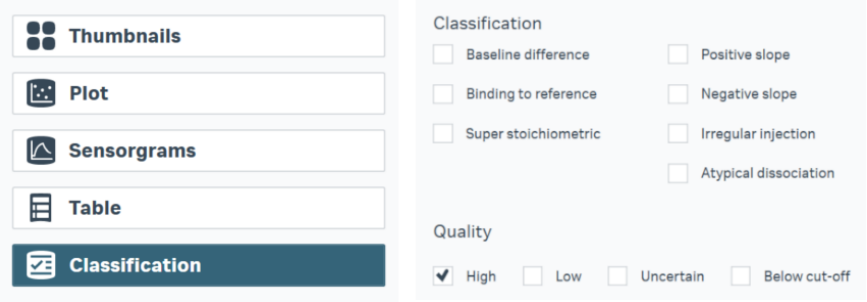
Classification panel

The ***Classification*** panel is available when ***Binder prediction*** is active (set to ***Use prediction***. The ***Classification*** panel shows the predicted classifications and quality of selected samples. All results that are predicted to be of ***Low*** quality always have at least one classification that motivates the quality prediction. The classifications and quality can be changed using the check boxes.

The section *Examples of ideal and non-ideal fragment profiles, on page 132* includes images of different binding profiles and how they are classified.

Note: *Compounds below cut-off do not receive any classifications. If the boundary is changed after prediction so that they become above cut-off, a new prediction is needed to obtain information about their quality and classifications.*

The heading of the ***Classification*** panel corresponds to the selection of samples. A line in a check box means that some, but not all, of the selected samples received the corresponding classification/quality. In the image below, one sample is selected. It is assigned ***Quality: High*** and has no classification.



The table below describes the possible settings of the ***Classification*** panel, using the default names of the classifications. The names presented in the ***Classification*** panel are the ones defined when creating the selected prediction model (see *Section 3.6.1 Panel description, on page 52*).

Setting	Description
Baseline difference	Large difference in the baseline levels between the current cycle and the next cycle.
Binding to reference	The remaining binding to the reference surface after the analyte injection is significant.
Super stoichiometric	The binding level is much higher than what can be expected.
Positive slope	The sensorgram has a significant positive slope during the analyte injection.
Negative slope	The sensorgram has a significant negative slope during the analyte injection.
Irregular injection	During the analyte injection: the sensorgram slope changes significantly, and/or has spikes or irregularities.
Atypical dissociation	During the dissociation phase: the sensorgram signal is significantly below the baseline at any time, there is a drift or an offset in the signal, and/or the dissociation is slow and the signal remains above the baseline for the duration of the dissociation phase.
Quality: High	The compound results were predicted to be of high quality.

Setting	Description
Quality: Low	The compound results were predicted to be of low quality. This can occur if, for example, it is super stoichiometric, binds to the reference or has an atypical sensorgram profile.
Quality: Uncertain	The prediction model is undecided on the quality of the compound results. User review is advised.
Quality: Below cut-off	The response levels are below the binding level cut-off.

6.6.6 Managing prediction models

The pretrained model for fragment binding level screen can be used, and potentially further trained, if it seems to predict your data well. It is also possible to create your own prediction model, which is done in the ***Prediction model*** workspace (see *Section 3.6 Prediction models, on page 51*).

Base your new model on the pretrained model if it seems to predict your data relatively well. If the generated data differ much from the data that the model was trained for, or if different adjustments are done than the recommended ones, it may be less suitable. In those cases, it is better to base the new model on an empty model. This is because an empty model quickly adapts to training, while the pretrained model needs more input to significantly change. An empty prediction model must be trained before it can be used to predict results.

Note: *The original pretrained models can only act as templates for other models, but not be used for prediction. This is to make sure that they are not accidentally overwritten upon training. In the predefined evaluation methods, copies of the original models are used instead.*

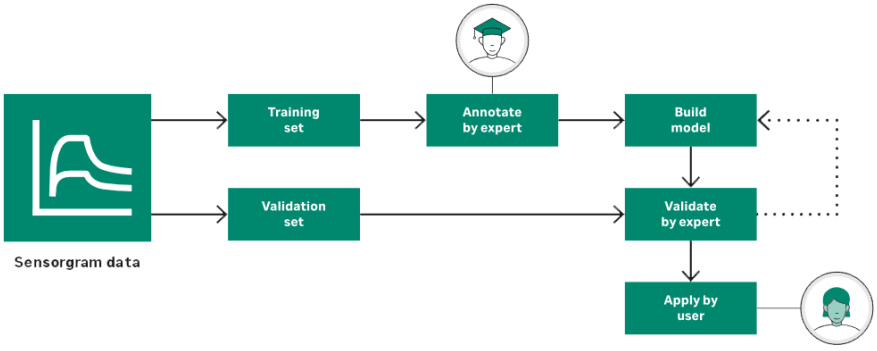
Train and save new version

The ***Train and save new version*** button, located in the ***Binder prediction*** setting, becomes activated when a change in classifications or quality state has been made. The prediction model is trained by clicking ***Train and save new version***, and the version number of the prediction model is incremented by one. Training a prediction model gradually customizes it to your preferences, and is always performed using the latest version of the prediction model.

Note: *Fragments set to **Uncertain** or **Below cut-off** are not included in training.*

Principle of Biacore Intelligent Analysis

The image and text below describe how the pretrained Biacore prediction model for binding level screen was originally created. The same approach may be used when training other models, to confirm that they perform as expected.



To train the model, data were divided into a training and a validation set. The training set was annotated by an expert who assigned classifications and quality levels to each sample. This input was used for training the model, which was then applied to the validation set.

The predictions of the validation set were examined by an expert and if the results were not satisfactory, the model continued to be trained with new data until the predictions of the validation set were at a satisfactory level.

6.7 Selecting data in Plot items

Introduction

This section describes how to select and manage data in ***Plot*** items. Individual points can be selected in the plot panel and the table panel. Select points to apply curve markers and classifications, or to exclude or include points in the evaluation session, and to display the sensorgrams from which the points are derived.

The thumbnail panel supports selection of groups but not individual points.

Selection in the *Thumbnails* panel

Group selection in the ***Thumbnails*** panel behaves differently according to whether ***Selection as toggle*** is checked in the thumbnail settings (see *Panel toolbar, on page 93*):

Selection as toggle	Description
Unchecked	Click a thumbnail to select the thumbnail. Any previously selected thumbnails will be deselected. Use **Shift-click** and **Ctrl-click** to select multiple thumbnails.
Checked	Click a thumbnail to toggle between selected and deselected. The status of other thumbnails is not affected.

Selected thumbnails are marked with a heavier border.

Selection in the *Plot* panel

Click on a point to select or deselect it.

Use ***Select area mode*** in the panel toolbar to select multiple points in one operation (see *General panel toolbar settings, on page 31*).

Selected points are shown as enlarged blue points. The corresponding rows in the table panel are selected, and corresponding sensorgrams are shown in the sensorgram panel. Classifications for selected points can be set in the ***Classification*** panel.

Click in the panel toolbar to deselect all selected points.

Selection in the *Sensorgrams* panel

Sensorgrams for points that are selected in the plot or table panels are displayed in the sensorgram panel (see *Section 6.1.4 Sensorgrams panel, on page 100*). Selected sensorgrams can be excluded from the evaluation or assigned curve markers, allowing management of subsets of data within the selected set of points. This feature is useful if you want to manage data points on the basis of sensorgram appearance rather than response levels.

Selection in the *Table* panel

Click on a row to select it. Use **Shift-click** and **Ctrl-click** to select multiple rows.

Selecting a row deselects any previously selected rows unless **Shift-click** or **Ctrl-click** is used.

Selected rows are highlighted in the table. The corresponding points in the plot panel are selected, and the corresponding sensorgrams are shown in the sensorgram panel.

Note: *Rows representing the currently selected group are highlighted in light green. This selection cannot be changed in the **Table** panel.*

Note: *You can use selection in the table to select points that are outside the currently selected group(s). The sensorgrams for the selected points will then be displayed in the sensorgram panel, but there will be no corresponding points selected in the plot panel.*

6.8 Calculated columns

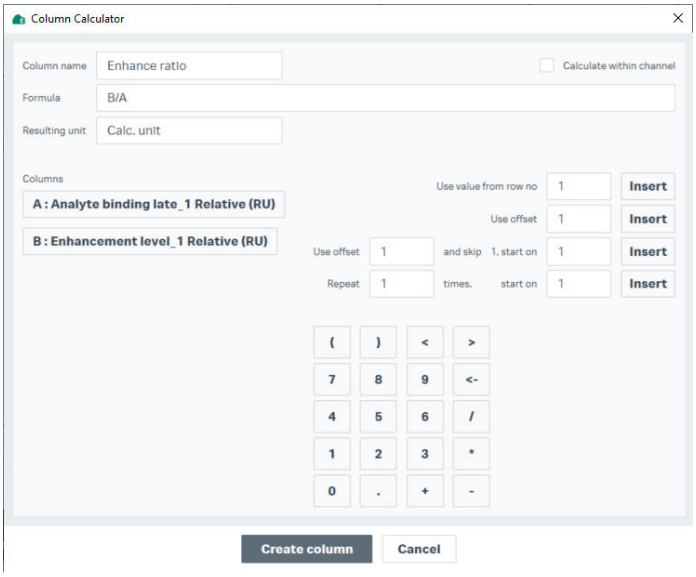
Introduction

Calculated columns can be added to tables, to hold the results of calculations based on numeric columns in the table. Calculated columns are available in the ***Table*** panel in ***Sensorgram***, ***Plot***, ***Concentration*** and ***Epitope binning*** items and in the ***Result table*** panel in ***Kinetics*** and ***Affinity*** items. The calculated values can be used as axis parameters of type ***Variable*** for the ***Plot*** item.

Adding calculated columns

Follow the steps below to add calculated columns to the table.

Step	Action
1	Click ***Table settings*** on the toolbar at the right of the workspace and select the ***Columns*** tab.
2	Click ***Column calculator***. ***Note:*** *The image below is from the **Plot** item. Fewer options are available in the other items.* *Result:* The column calculator dialog is displayed.

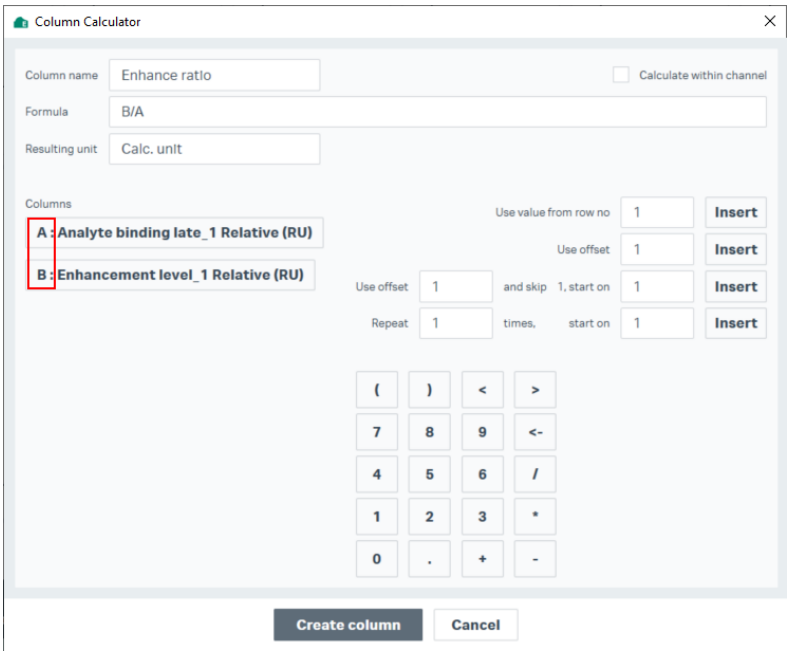


Step	Action
3	Provide a name for the calculated column. By default, the column will be named as the calculation formula.
4	For ***Plot*** items, check ***Calculate within flow cell*** or ***Calculate within channel*** if the calculation is to be performed using only values from the same flow cell (1-channel) or channel (8-channel).
5	Enter a formula using one or more of the following: **a.** Column identifiers (A, B, C, etc,) as listed in the dialog (see *Column identifiers, on page 149*). **b.** Inserted functions (see *Referencing functions, on page 150*), only available in ***Plot*** items. **c.** Arithmetical operators and numerical values entered with the keypad. The formula can also be typed directly in the ***Formula*** field.
6	Enter a unit for the calculated column in ***Resulting unit***. The unit will be shown in the axis label when the column is used as a plot axis.
7	Click ***Create column***. *Result:* The calculated column will be added to the table.

Column identifiers

Columns that can be used in the calculation are listed with an alphabetical identifier in the ***Column calculator*** dialog. Columns are referenced in the calculation formula by this identifier.

Note: *The image below is from the* ***Plot*** *item. Fewer options are available in the other items.*

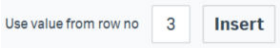
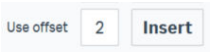
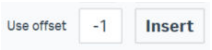


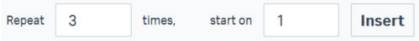
Click a column button or type the identifier letter to add the identifier to the formula. Identifiers are case-sensitive.

Note: *Removing columns from the table after defining calculated columns is not recommended. If columns are added to or removed from the table, column identifiers will be reassigned but existing calculated column expressions will still use the original identifiers. Calculated values are however not recalculated.*

Referencing functions

Calculations using only column identifiers and arithmetical operators are performed for each row in the table. Referencing functions allow calculations to use column values from different rows in the ***Plot*** item. To add a referencing function, click a column button, enter the value(s) in the text field(s) to the right of the column buttons, and click ***Insert***. It is also possible to write them directly in the ***Formula*** field using the syntax described below.

Function	Syntax[1]	Description
Use value from row no. x	A(x)	Inserts the fixed value from row x into the formula. *Example 1*: A(3) uses the value on row 3.  *Example 2:* A-A(3) subtracts the value on row 3 from the current row. **Analyte 1 Stability early** / **A(3)** / **A-A(3)** 10 / 30,00 / -20,00 20 / 30,00 / -10,00 30 / 30,00 / 0,000 40 / 30,00 / 10,00 50 / 30,00 / 20,00 60 / 30,00 / 30,00
Use offset x	A(O;x) [2]	Inserts the value from the (current + x) row into the formula. *Example 1:* A(O;2) uses the value from two rows below the current row.  *Example 2:* A(O;-1) uses the value from one row above the current row.  *Example 3:* A/A(O;2) divides the current row value by the value from two rows below. **Analyte 1 Stability early** / **A(O;2)** / **A(O;-1)** / **A/A(O;2)** 10 / 30.00 / / 0.3333 20 / 40.00 / 10.00 / 0.5000 30 / 50.00 / 20.00 / 0.6000 40 / 60.00 / 30.00 / 0.6667 50 / / 40.00 / 60 / / 50.00 /

Function	Syntax[1]	Description
Use offset x and skip x, start on y	A(OS;x;y)	The calculation starts on the specified row y, inserts the value from the (current + x) row and then skips x number of rows before repeating the procedure. The skipping number is always the same as the offset number. Rows that are skipped are empty in the calculated column. *Example:* A(OS;2;1) starts on row 1 and uses the value from 2 rows below. Then it skips 2 rows before repeating the procedure. **Analyte 1 Stability early** / **A(OS;2;1)** 10 — 30,00 20 30 40 — 60,00 50 60
Repeat x times, start on y	A(RS;x;y)	The calculation starts on the specified row y and repeats the value from that row x times. Then the procedure starts over on row x+1. *Example:* A(RS;3;1) starts on row 1 and uses that value 3 times. Then it moves to row 4 and uses that value 3 times.  **Analyte 1 Stability early** / **A(RS;3;1)** 10 — 10,00 20 — 10,00 30 — 10,00 40 — 40,00 50 — 40,00 60 — 40,00

[1] "A" represents any column identifier. "x" and "y" indicate entered parameters.
[2] Uppercase letter O, not the numeral zero.

Automatic column recalculation

Calculated columns are recalculated automatically under certain circumstances when the table content is changed, according to the following table.

Change in table content	Recalculation
Change in cell values	Yes
Addition or removal of rows	Yes
Re-sorting table rows[1]	No
Removal of referenced columns	No

[1] The sort order that applies for a calculated column is shown in the tooltip for the column.

Calculated columns that are not automatically recalculated retain their original correct numerical values.

Calculated columns cannot be updated after a change in table content that does not cause recalculation. New calculated columns must be defined to reflect the changed table content.

Calculated columns in evaluation methods

Table columns and sort order that applied when calculated columns were defined are recorded in evaluation methods and applied when the method is used. In this way, column calculations in an evaluation method will be correctly applied, provided that the referenced columns are available in the table.

7 Concentration items

About this chapter

This chapter describes how to use ***Concentration*** items. Evaluation with ***Concentration*** items requires that the ***Concentration & Potency*** extension is active.

In this chapter

7.1 Overview

Introduction

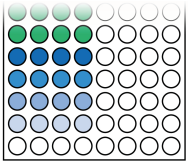
Concentration items are used for evaluating analyte concentration in unknown samples by comparing the response or sensorgram slope with that of calibration solutions analyzed in the same way. Calibration curves may be repeated at intervals during the run if desired, to adjust the results for any drift in the assay. Repeated calibration curves can be used to create *calibration trends*, to compensate for drift in the assay performance by interpolating calibration data between measured cycles. Control samples repeated at intervals check that the assay performance is sufficiently consistent throughout the assay.

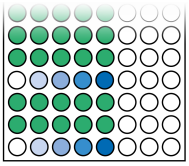
Available predefined evaluation models support direct binding assays (DBA), inhibition in solution assays (ISA) and surface competition assays.

Note: *Calibration-free concentration analysis (CFCA), available in some other Biacore systems, is not currently supported in Biacore Insight Evaluation software. CFCA runs in files imported from Biacore T200 and Biacore S200 cannot be evaluated in Biacore Insight Evaluation software.*

Experimental formats

Concentration assays may be run using serial or parallel mode, referring to the layout of calibrants and samples in the microplate. The illustrations show the disposition of 5 calibration solutions (blue) and several samples (green) for an 8 series system in the two modes.

Mode	Description	Illustration
Serial	Serial mode is suitable for both 1-channel and 8-channel systems. Concentration series for calibration solutions are run in multiple cycles within one channel. Samples are evaluated using the calibration curve from the same flow cell (1-channel) or channel (8-channel) as the sample. For 8-channel systems, samples are distributed along columns in the microplate.	

Mode	Description	Illustration
Parallel	Parallel mode is only possible in 8-channel systems. Concentration series for calibration solutions are distributed across rows in the microplate, and run in one cycle for each curve. Samples are evaluated using the calibration curve from the same set of channels as the sample. A ***Channel normalization*** assay step is included in the run method, to compensate for variations in response from different channels (see *Channel normalization, on page 156*). (This cycle is not shown in the accompanying illustration.)	

Channel normalization

For 8-channel instruments, small variations in the analyte response between channels can affect the precision of concentration measurements performed in parallel mode. To compensate for this, parallel concentration runs should include a ***Channel normalization*** cycle that injects identical analyte samples over all channels. Responses from other cycles (calibration, sample and control) are adjusted using a normalization factor for each channel, according to:

$$\text{Adjusted response} = \text{Response} \times \frac{\text{Channel normalization average}}{\text{Channel normalization response}}$$

This adjustment is applied automatically when ***Data grouping*** is set to ***Parallel*** in the ***Concentration*** item, provided that the analysis step ***Channel normalization*** is included in the evaluation. To turn channel normalization off, exclude the channel normalization analysis step or cycles in the ***Select sensorgrams*** panel (see *Workspace settings, on page 30*).

Channel normalization is only supported for parallel concentration assays.

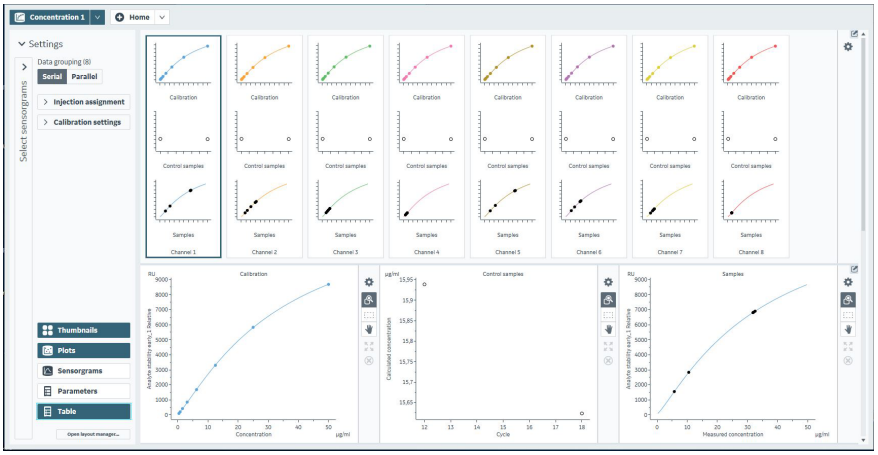
7.2 The Concentration workspace

In this section

7.2.1 Introduction

The ***Concentration*** item workspace holds up to 5 panels in addition to the ***Settings*** panel at the left of the workspace.

Note: *Some panels may lie outside the screen area in the default view. Scroll the workspace or hide unwanted panels as required.*



Panel	Content
Thumbnails	Displays the data in the evaluation grouped based on flow cell (1-channel data) or according to the ***Data grouping*** setting (8-channel data). Thumbnails showing calibration curves, control samples, and samples overlaid on calibration curves, are shown for each group. See *Section 7.2.2 Thumbnails panel, on page 160* for details.
Plots	Displays three sub-panels with calibration, control and samples plots for the currently selected group(s). See *Section 7.2.3 Plots panel, on page 163* for details.
Sensorgrams	Displays sensorgrams for points selected in the ***Plots*** panel. Points may be selected from any of the sub-panels. See *Section 7.2.4 Sensorgrams panel, on page 167* for details.
Parameters	Displays the fitting parameters for all calibration curves in the evaluation. See *Section 7.2.5 Parameters panel, on page 170* for details.

Panel	Content
Table	Displays the details for all cycles in the evaluation. Cycles in the selected groups are highlighted. See *Section 7.2.6 Table panel, on page 171* for details.

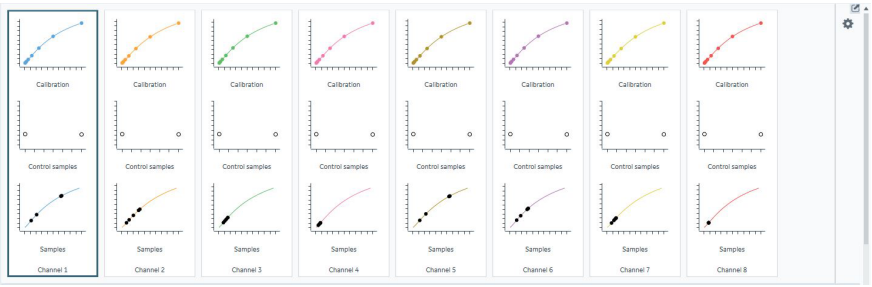
Use the buttons at the bottom of the ***Settings*** panel to show or hide selected panels.

The panels are described in more detail in the following subsections.

7.2.2 Thumbnails panel

Panel description

The ***Thumbnails*** panel displays thumbnails of the data grouped based on flow-cell (1-channel data) or according to the ***Data grouping*** setting (8-channel data). For each group, separate thumbnail plots are shown for calibration, control and sample data. You may need to scroll the panel display to see all thumbnails.



Select one or more thumbnails to display the contents in the ***Plots*** panels. Use **Shift-click** and **Ctrl-click** to select multiple thumbnails. Selected thumbnails are marked by a heavier border.

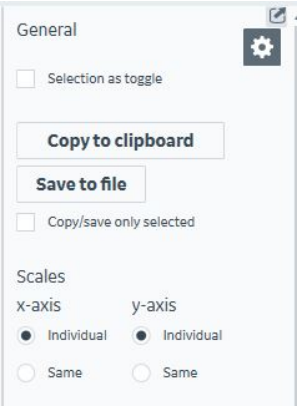
Note: *Calibration curves are plotted as response (or slope) against expected concentration. Samples are plotted as response (or slope) against measured concentration. Control samples are plotted as calculated concentration against cycle.*

Deselect ***Thumbnails*** in the ***Settings*** panel at the left to collapse the thumbnails to buttons. Data groups can be selected with these buttons even when the thumbnail content is not displayed.

Panel toolbar

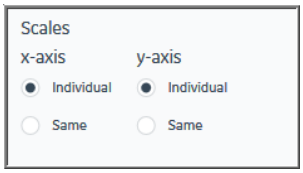
Click ⚙ ***Thumbnail settings*** on the panel toolbar to access display settings for the thumbnails. Settings are applied to all thumbnails. The settings are divided into two sections, ***General*** and ***Scale***.

General section



Setting	Description
Selection as toggle	If this option is checked, clicking on a thumbnail will toggle between selected and deselected. When the option is not selected, clicking will only select the thumbnail. ***Note:*** *When the option is checked, using* **Shift-click** *will toggle the status of all thumbnails in the range covered.*
Copy to clipboard	Copies thumbnails to the Windows clipboard as a collection of graphical objects. The thumbnails can be pasted into programs that support pasting for collections of graphical objects. Check ***Copy/save only selected*** to copy only selected thumbnails. If this option is not checked, all thumbnails will be copied.
Save to file	Saves thumbnails as illustrations in ***.png*** (Portable Network Graphics) format. Each thumbnail is saved to a separate file. File names are constructed from the thumbnail label with an added serial number to prevent duplicate names. Check ***Copy/save only selected*** to save only selected thumbnails. If this option is not checked, all thumbnails will be saved.

Scales section



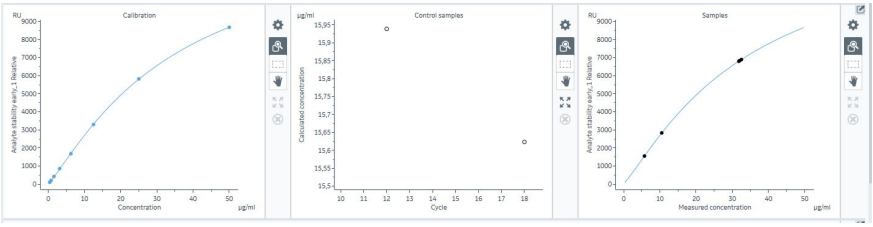
Setting	Description
x-axis ***y-axis***	Determines how the axes are scaled in the thumbnails. • ***Individual*** scales the thumbnails for each data group separately according to the range of values in the corresponding plot. • ***Same*** scales all thumbnails of the same type to the same scale, determined by the range of values in all data groups combined.

Calibration thumbnails are scaled according to the range of calibration data. Sample thumbnails are scaled according to the range covered by the corresponding calibration curve, taking any excluded calibration points into account.

7.2.3 Plots panel

Panel description

The ***Plots*** panel displays separate plots for the calibration curve, control samples, and sample points overlaid on the calibration curve.



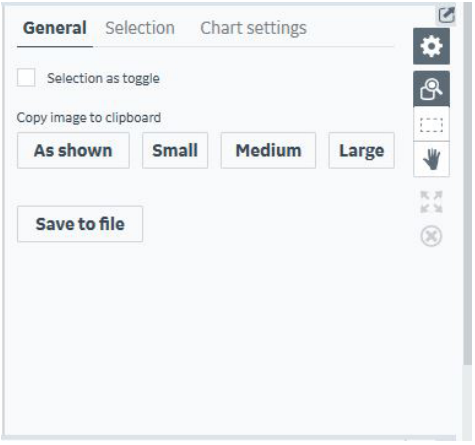
Click on a point in any of the three plots to select it. Selection mode is determined by the ***Selection as toggle*** setting on the ***General*** tab under ***Plot settings*** (see *Selection in the Plots panel, on page 179*). Selected points are shown as enlarged blue points, and the sensorgrams from selected points are shown in the sensorgram panel.

Panel toolbar

Click ⚙ ***Chart settings*** on the panel toolbar to access display settings for the panel. Each of the three plot panels has a separate panel toolbar.

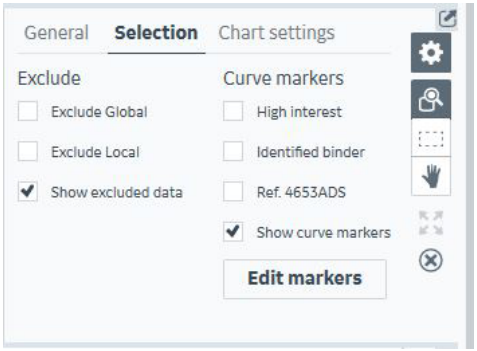
The separate tabs in the chart settings are described below. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.

General tab



Setting	Description
Selection as toggle	Determines the point selection mode (see *Selection in the Plots panel, on page 179*).
Copy image to clipboard	Copies the plot panel to the Windows clipboard. Choose one of three sizes or ***As shown***. ***Note:*** ***Small***, ***Medium*** *and* ***Large*** *copies use default image proportions.* ***As shown*** *retains the proportions as displayed on the screen.*
Save to file	Saves the plot panel as an illustration in selectable format.

Selection tab



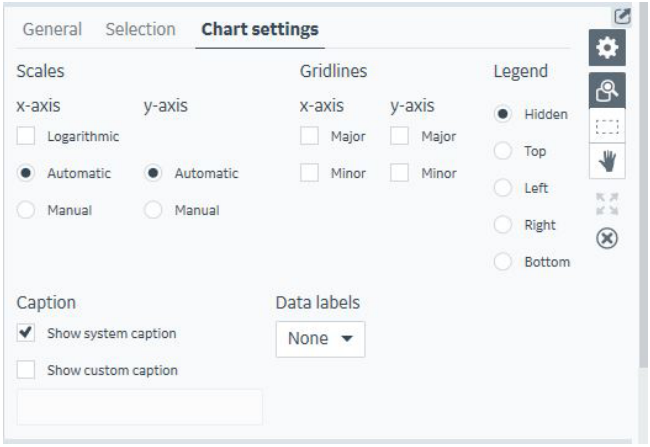
Note: *The curve markers shown here are examples, for illustration purposes only.*

Options on this tab apply to selected plot points. See *Section 6.7 Selecting data in Plot items, on page 146* for details of how to select points.

Setting	Description
Exclude global	Check this option to exclude the selected points and the sensorgrams from which they are derived from all evaluation items, including those already created. If excluded points are selected, the option will be checked. Remove the checkmark to include the points again.

Setting	Description
Exclude local	Check this option to exclude the selected points and the sensorgrams from which they are derived from the current evaluation item only. If excluded points are selected, the option will be checked. Remove the checkmark to include the points again.
Show excluded data	Check this option to show excluded data in the plot panel. Excluded points are shown as gray crosses.
Curve markers	Check the required markers to apply the markers to the selected points. See *Section 3.4 Curve markers, on page 44* for more details.
Show curve markers	Check this option to show curve markers in the plot, thumbnail, and sensorgram panels.
Edit markers	Choose this option to manage curve markers. See *Section 3.4 Curve markers, on page 44* for details.

Chart settings tab

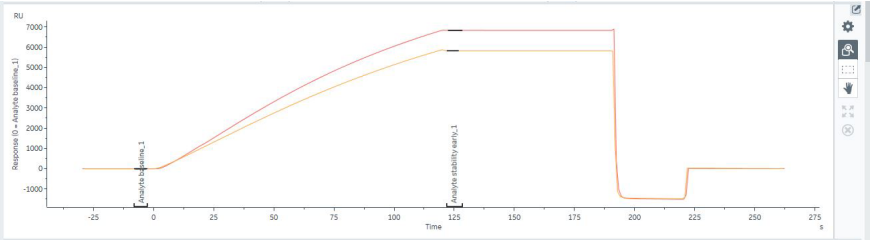


Setting	Description
Scales	Choose ***Automatic*** or ***Manual*** scales for the axes. For ***Manual*** scaling, enter minimum and maximum values. Choose ***Logarithmic*** to use a logarithmic axis scale. ***Note:*** *Zero or negative values cannot be displayed on a logarithmic scale.* *Logarithmic scales can only be used for the x-axis in the* ***Calibration*** *and* ***Samples*** *plots.*
Gridlines	Choose whether to show major and/or minor gridlines. Gridline spacing is determined according to the scale range.
Legend	Displays a legend identifying calibration curves by color. Choose the position of the legend in the panel.
Caption	Displays a caption in the panel. Choose whether to include the system caption and/or a custom caption with text as entered.
Data labels	Displays a label for each point in the plot. Choose the property to use as label text.

7.2.4 Sensorgrams panel

Panel description

The ***Sensorgrams*** panel shows the sensorgrams for points that are selected in the ***Plots*** panel. The panel is empty when no points are selected.

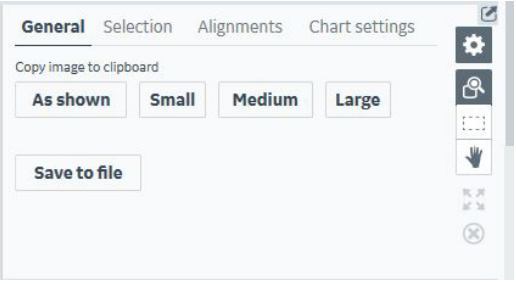


Panel toolbar

Click ***Chart settings*** on the panel toolbar to access display settings for the panel.

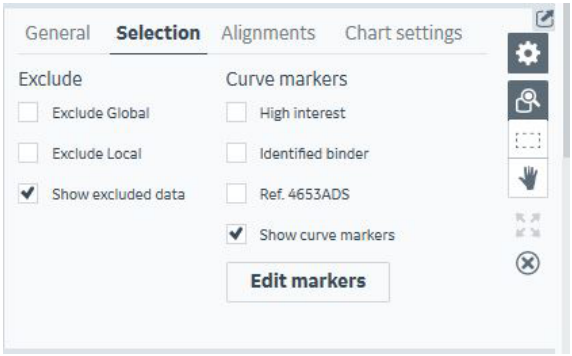
The separate tabs in the chart settings are described below. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.

General tab



Setting	Description
Copy image to clipboard	Copies the sensorgram panel to the Windows clipboard. Choose one of three sizes or ***As shown***. ***Note:*** ***Small***, ***Medium*** *and* ***Large*** *copies use default image proportions.* ***As shown*** *retains the proportions as displayed on the screen.*
Save to file	Saves the sensorgram panel as an illustration in selectable format.

Selection tab

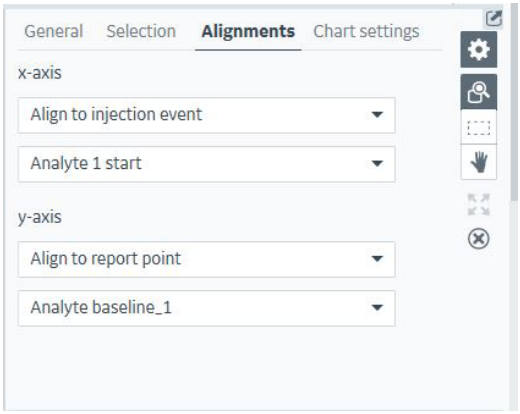


Note: *The curve markers shown here are examples, for illustration purposes only.*

Options on this tab apply to selected sensorgrams. See *Section 5.3 Selecting data in Sensorgram items, on page 88* for details on how to select sensorgrams.

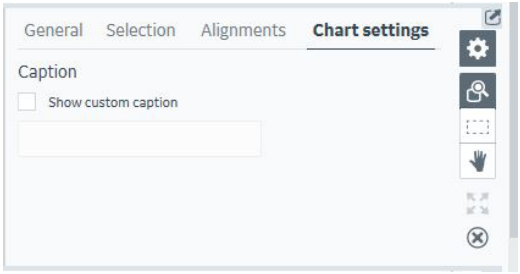
Setting	Description
Exclude global	Check this option to exclude the selected sensorgrams from all evaluation items, including those already created. If excluded sensorgrams are selected, the option will be checked. Remove the checkmark to include the sensorgrams again. Excluding sensorgrams removes any sensorgram adjustments and/or table calculations that may have been applied.
Exclude local	Check this option to exclude the selected sensorgrams from the current evaluation item only. If excluded sensorgrams are selected, the option will be checked. Remove the checkmark to include the sensorgrams again.
Show excluded data	Check this option to show excluded data in the sensorgram panel. Excluded sensorgrams are shown as gray lines.
Curve markers	Check the required markers to apply the markers to the selected sensorgrams. See *Section 3.4 Curve markers, on page 44* for more details.
Show curve markers	Check this option to show curve markers in the sensorgram and thumbnail panels.
Edit markers	Choose this option to manage curve markers. See *Section 3.4 Curve markers, on page 44* for details.

Alignments tab



Setting	Description
x-axis ***y-axis***	Choose how sensorgrams should be aligned in the display. The x- and y-axes can be aligned independently to an injection event or a report point. The alignment point will be set to zero in the display.

Chart settings tab



Setting	Description
Caption	Includes a custom caption in the panel. Enter the caption text.

7.2.5 Parameters panel

Panel description

The ***Parameters*** panel shows the fitting parameters for all calibration curves in the evaluation item. See *Appendix A.3 Other fitting models, on page 296* for fitting parameters and *Chi-square, on page 264* for chi-square definition.

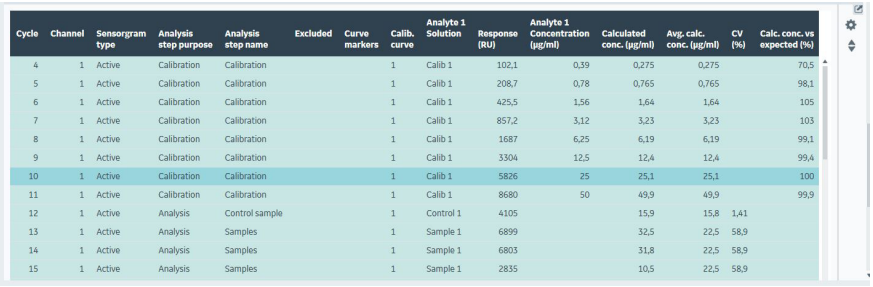
Calibration curve	Rhi	Rlo	A1	A2	Chi²
1	1,423e4	56,75	34,48	1,188	704,4
2	1,462e4	56,28	36,98	1,188	706,6
3	1,455e4	57,53	34,71	1,172	678,8
4	1,407e4	63,60	33,70	1,211	839,9
5	1,444e4	58,49	36,44	1,189	753,8
6	1,435e4	58,62	33,94	1,184	803,1
7	1,420e4	55,03	36,96	1,187	805,1
8	1,416e4	65,19	35,11	1,193	1150

There are no settings in the ***Parameters*** panel.

7.2.6 Table panel

Panel description

The table panel lists parameter values for all points included in the concentration item. Rows for points in the currently selected group are highlighted in light green, and rows for selected points are highlighted in a darker shade.



Cycle	Channel	Sensorgram type	Analysis step purpose	Analysis step name	Excluded	Curve markers	Calib. curve	Analyte 1 Solution	Response (RU)	Analyte 1 Concentration (µg/ml)	Calculated conc. (µg/ml)	Avg. calc. conc. (µg/ml)	CV (%)	Calc. conc. vs expected (%)
4	1	Active	Calibration	Calibration			1	Calib 1	102,1	0,39	0,275	0,275		70,5
5	1	Active	Calibration	Calibration			1	Calib 1	208,7	0,78	0,765	0,765		98,1
6	1	Active	Calibration	Calibration			1	Calib 1	425,5	1,56	1,64	1,64		105
7	1	Active	Calibration	Calibration			1	Calib 1	857,2	3,12	3,23	3,23		103
8	1	Active	Calibration	Calibration			1	Calib 1	1687	6,25	6,19	6,19		99,1
9	1	Active	Calibration	Calibration			1	Calib 1	3304	12,5	12,4	12,4		99,4
10	1	Active	Calibration	Calibration			1	Calib 1	5826	25	25,1	25,1		100
11	1	Active	Calibration	Calibration			1	Calib 1	8680	50	49,9	49,9		99,9
12	1	Active	Analysis	Control sample			1	Control 1	4105		15,9	15,8	1,41	
13	1	Active	Analysis	Samples			1	Sample 1	6899		32,5	22,5	58,9	
14	1	Active	Analysis	Samples			1	Sample 1	6803		31,8	22,5	58,9	
15	1	Active	Analysis	Samples			1	Sample 1	2835		10,5	22,5	58,9	

Click on a column header to sort the table by the content of that column.

Concentration results

The table columns listed below contain results from the concentration evaluation. Depending on the ***Columns*** setting (see *Columns tab, on page 174*), some columns may be hidden.

Concentration values are not reported for samples that lie outside the range of the calibration curve.

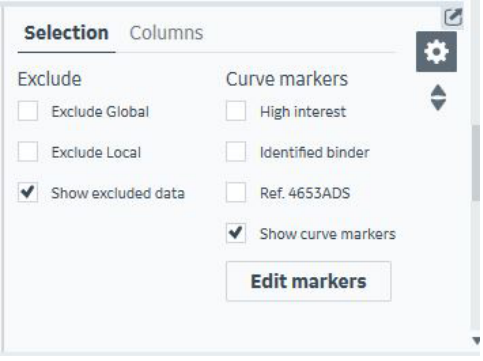
Column	Description
Calib. curve	Calibration curve number used for the calibrant or sample in the current row. Shown for samples as ***Trends*** if calibration trends are used.
<Command name> concentration	Analyte concentration as entered in the method. Analyte concentration is required for calibrants and may also be entered for samples (representing an expected concentration).
<Command name> dilution	Analyte dilution factor as entered in the method (only relevant for samples and controls). The dilution factor is taken as 1 if no dilution is entered in the method.
Response*/*Normalized response	The response at the report point used for concentration measurement. Shown as ***Normalized response*** if channel normalization has been applied.
Measured conc.	Analyte concentration as measured from the calibration curve.

Column	Description
Calculated conc.	Measured analyte concentration multiplied by the dilution factor, to give the concentration in the original undiluted sample.
Avg. calc. conc.	Average calculated concentration for samples that are analysed in replicate.
CV (%)	Coefficient of variation (%) for replicate samples.
Calc. conc. vs expected (%)	Calculated concentration as a percent of the concentration entered in the method.
Avg. vs expected (%)	Average concentration as a percent of the concentration entered in the method.

Panel toolbar

Setting	Description
Table settings	Opens the display settings for the panel. Tabs in the display settings are described below.
Sort on selection	Groups selected rows at the top or bottom of the table. Click repeatedly to switch between top and bottom of the table.

Selection tab

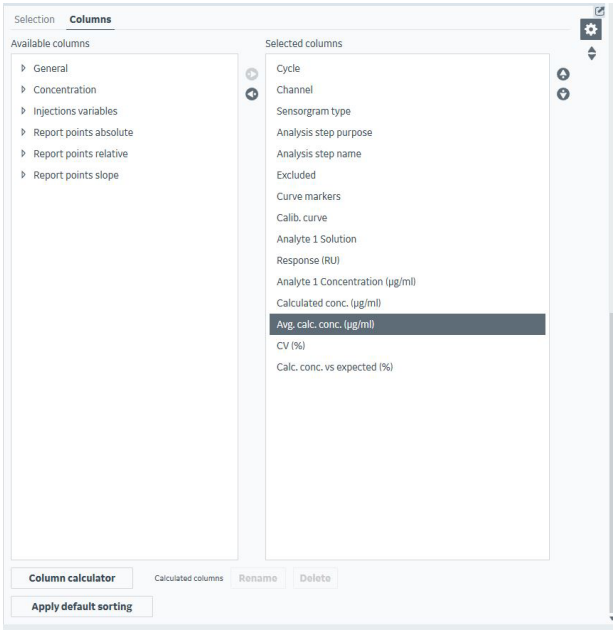


Note: *The curve markers shown here are examples, for illustration purposes only.*

Options on this tab apply to selected table rows. See *Section 7.5 Selecting data in concentration items, on page 179* for details of how to select points.

Setting	Description
Exclude global	Check this option to exclude the selected points and the sensorgrams from which they are derived from all evaluation items, including those already created. If excluded sensorgrams are selected, the option will be checked. Remove the checkmark to include the sensorgrams again.
Exclude local	Check this option to exclude the selected points and the sensorgrams from which they are derived from the current evaluation item only. If excluded sensorgrams are selected, the option will be checked. Remove the checkmark to include the sensorgrams again.
Show excluded data	Check this option to show excluded points and sensorgrams in gray, and to list excluded data in the table. Excluded sensorgrams are identified in the table in the ***Excluded*** column. If this option is not checked, sensorgrams and corresponding table rows are hidden.
Curve markers	Check the required markers to apply the markers to the selected points and sensorgrams. See *Section 3.4 Curve markers, on page 44* for more details.
Edit markers	Choose this option to manage curve markers. See *Section 3.4 Curve markers, on page 44* for details.

Columns tab



Move columns between ***Available columns*** and ***Selected columns*** using the left and right arrow buttons to control the information displayed in the table. Select a column in the ***Selected columns*** list and use the up and down arrow buttons to change the column display order.

Note: *Columns that are not selected cannot be used for axis variables in the plot (see Axis settings, on page 109). If you remove columns from the table that are used as axis settings, the thumbnails and plot will be adjusted.*

Click ***Apply default sorting*** to restore the default sort order for table rows if you have changed the sort order.

Note: *Applying default sorting does not affect the order of selected columns.*

7.3 Concentration item settings

Introduction

This section describes the available settings in the ***Settings*** panel at the left of the concentration workspace. The ***Select sensorgrams*** panel is described in *Workspace settings, on page 30*.

Data grouping

The ***Data grouping*** setting determines how the data is divided into groups for 8-channel systems. The number of groups is shown in parentheses in the ***Data grouping*** setting header. Data from 1-channel systems are automatically grouped as ***Serial*** (see description of ***Serial*** below) without the ***Data grouping*** option visible.

The following ***Data grouping*** settings are available in a ***Concentration*** item. Use the setting that corresponds to how the experiment was set up (see *Experimental formats, on page 155*):

Setting	Description
Serial	Calibration curves are constructed from unbroken series of cycles with assay step ***Calibration*** in the same flow cell (1-channel data) or channel (8-channel data). Samples are analyzed using the calibration curve in the same flow cell/channel.
Parallel	Calibration curves are constructed from calibrants with the same calibrant name in different channels in cycles with assay step ***Calibration***.

Injection assignment

The ***Injection assignment*** setting determines which injections should be used for variable information and response values respectively.

Setting	Description
Use variable information from	Variable parameters from the specified injection command will be used as sample parameters in the evaluation.

Calibration settings

Calibration settings determine how the calibration curve is constructed. Only cycles with ***Analysis step purpose*** set to ***Calibration*** are used to construct the calibration curve.

Setting	Description
Report point	Select the report point to use for concentration analysis. The same report point will be used in all cycles.
Response type	Select the response type (relative response or sensorgram slope).
Calibration curve	Select which calibration curve to apply to a given sample in runs where calibration curves are repeated. See *Section 7.4 Calibration curve options, on page 177* for description of the options.
Fitting function	Select the fitting function used to construct the calibration curve. See *Appendix A.3 Other fitting models, on page 296* for details of the available models.

7.4 Calibration curve options

Settings

The following settings are available:

Setting	Description
Preceding	The closest preceding calibration curve will be used for each sample. If there is no preceding calibration curve, the nearest following curve is used.
Average	An average of all calibration curves will be used for all samples. Applies within flow cells (1-channel data) or channels (8-channel data) in serial mode.
Calibration trends	Each sample will be calculated from an individual calibration curve obtained by interpolation according to calibration trends. See following subsections for details. Calibration trends are not available in parallel mode.

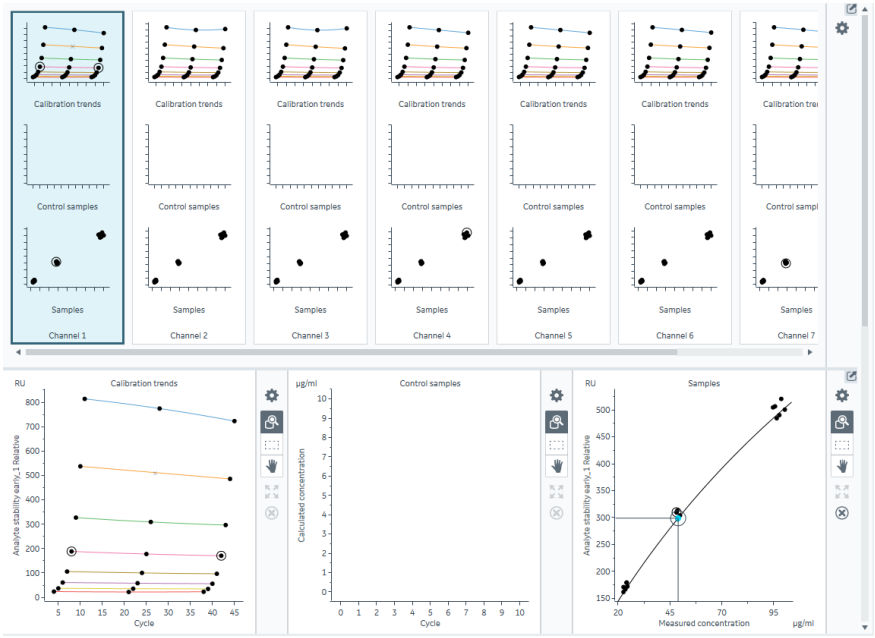
Calibration trends

Calibration trends can compensate for systematic drift in the calibration curve by constructing an individual calibration curve for each cycle in the run. Calibration points are plotted against cycle number, and trend lines are fitted through each set of points with the same calibrant concentration. Individual calibration curves for each cycle are constructed by interpolation using the trend line values.

Trend lines are fitted to the measured calibration points using a linear function for trends with two points and a polynomial function (see *Appendix A.3 Other fitting models, on page 296*) for trends with three or more points. If calibration points at the ends of the trend lines are excluded, the trend lines will not be extrapolated.

When ***Calibration trends*** are selected, the calibration curve thumbnails and plots are replaced by calibration trends.

Calibration trends are not extrapolated. Therefore, concentrations cannot be calculated for sample cycles before the first calibration curve or after the last calibration curve (calibration trend range). Consequently, averages and CV (%) are not calculated for samples having multiplicates both on the inside and on the outside of the calibration trend range.



Individual calibration curves for selected points are displayed in the ***Samples*** plot.

7.5 Selecting data in concentration items

Introduction

This section describes how to select and manage data in ***Concentration*** items. Individual points can be selected in the plots panel and the table panel. Select points in the ***Plots*** panel to apply curve markers and exclude or include points in the evaluation session, and to display the sensorgrams from which the points are derived.

Selection in the *Thumbnails* panel

Group selection in the ***Thumbnails*** panel behaves differently according to whether ***Selection as toggle*** is checked in the thumbnail settings (see *Panel toolbar, on page 160*):

Selection as toggle	Description
Unchecked	Click a thumbnail to select the thumbnail. Any previously selected thumbnails will be deselected. Use **Shift-click** and **Ctrl-click** to select multiple thumbnails.
Checked	Click a thumbnail to toggle between selected and deselected. The status of other thumbnails is not affected.

Selected thumbnails are marked with a heavier border.

Selection in the *Plots* panel

Point selection in the ***Plots*** panel behaves differently according to whether ***Selection as toggle*** is checked in the plot settings (see *Panel toolbar, on page 93*):

Selection as toggle	Description
Unchecked	Within a given subpanel, only one point can be selected by clicking on the point. Clicking on a second point will deselect the previously selected point. Use the ***Area select*** mode to select multiple points in one plot by dragging around them (see *General panel toolbar settings, on page 31*). Clicking on an already selected point has no effect.
Checked	Clicking on successive points will add the points to the selection. Clicking on an already selected point will deselect that point.

Points can be selected for sensorgram display from different subpanels in the same or different data groups. However, excluding or including points or setting curve markers will only apply to points in the subpanel where the operation is performed.

Note: *The **Selection as toggle** setting applies separately to each of the three plot panels.*

The ***Select area mode*** in the panel toolbar can be used to select multiple points in one operation (see *General panel toolbar settings, on page 31*). This feature is independent of the ***Selection as toggle*** setting.

Selected points are shown as enlarged blue points. The corresponding rows in the table panel are selected.

Click in the panel toolbar to deselect all selected points.

Selection in the *Sensorgrams* panel

Sensorgrams for points that are selected in the plot or table panels are displayed in the sensorgram panel (see *Section 6.1.4 Sensorgrams panel, on page 100*). Selected sensorgrams can be excluded from the evaluation or assigned curve markers, allowing management of subsets of data within the selected set of points. This feature is useful if you want to manage data points on the basis of sensorgram appearance rather than response levels.

Selection in the *Table* panel

Click on a row to select it. Use **Shift-click** and **Ctrl-click** to select multiple rows.

Selecting a row deselects any previously selected rows unless **Shift-click** or **Ctrl-click** is used.

Selected rows are highlighted in the table. The corresponding points in the plot panel are selected, and the corresponding sensorgrams are shown in the sensorgram panel.

Note: *Rows representing the currently selected group are highlighted in light green. This selection cannot be changed in the **Table** panel.*

8 Epitope binning items

About this chapter

This chapter describes how to use ***Epitope binning*** items. Evaluation with ***Epitope binning*** items requires that the ***Epitope binning*** extension is active.

In this chapter

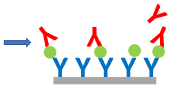
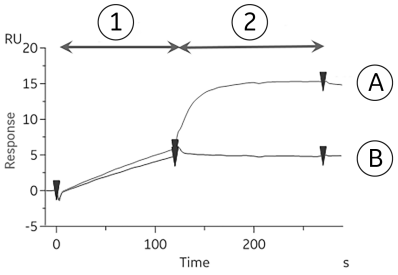
8.1 Overview

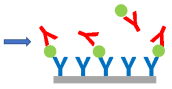
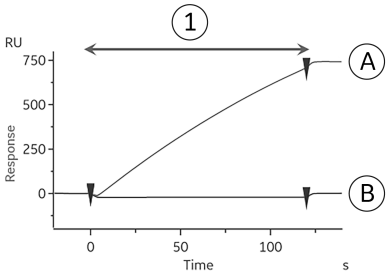
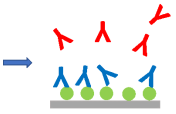
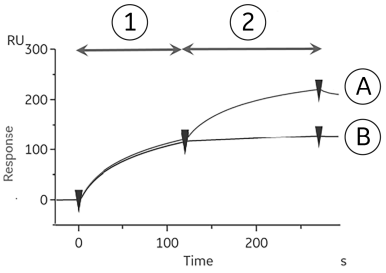
Introduction

Epitope binning is used to characterize binding of monoclonal antibodies to an antigen. Antibodies specific to the same antigen are tested in a pairwise combinatorial manner to assess whether or not they block one another's binding to the antigen. Antibodies that share a common blocking profile can be assumed to bind to the same epitope, or overlapping epitopes, and are binned together.

Experimental formats

Which assay format to choose will depend on the nature of the reagents and the available amount of interactants. The most commonly used assay formats for epitope binning are sandwich, premix and tandem, described below.

Assay format	Description	Example sensorgram
Sandwich	The first antibody is attached to the surface. Antigen is then injected over the first antibody, followed by injection of the second antibody. 	 **1** Antigen injection **2** 2nd antibody injection **A** Non-blocking **B** Blocking

Assay format	Description	Example sensorgram
Premix	The first antibody is attached to the surface. The premixed solution with antigen and second antibody is then injected over the first antibody. 	 **1** Antigen injection and 2nd antibody injection injected together **A** Non-blocking **B** Blocking
Tandem	The antigen is attached to the surface. The first antibody is then injected over the antigen, followed by the second antibody. 	 **1** 1st antigen injection **2** 2nd antigen injection **A** Non-blocking **B** Blocking

Capture

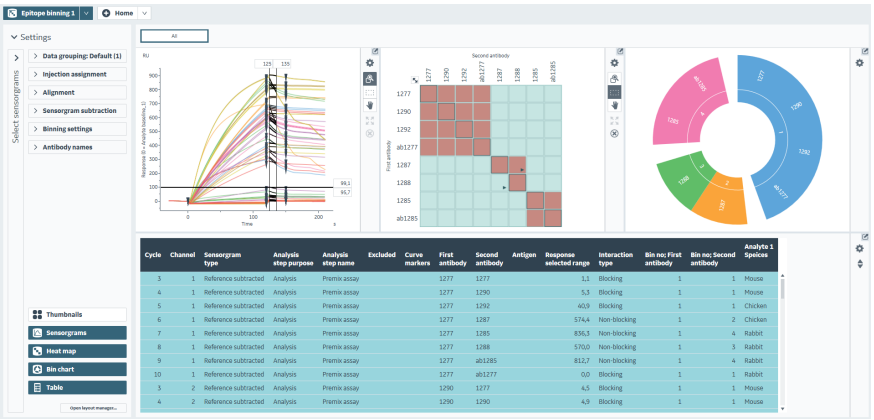
The assay formats can be run with capture of the first antibody, which allows for larger matrices to be tested. However, capture requires thorough blocking of remaining free binding sites on the capture molecule after capture of the first antibody, to prevent the second antibody from binding to the surface.

8.2 The Epitope binning workspace

In this section

8.2.1 Introduction

The ***Epitope binning*** item workspace holds up to 5 panels in addition to the ***Settings*** panel at the left of the workspace. One interaction between a certain first antibody and a certain second antibody is represented by one sensorgram, one cell in the heat map and one row in the table.



Panel	Content
Thumbnails	Displays sensorgram data grouped according to the ***Data grouping*** setting. See *Section 8.2.2 Thumbnails panel, on page 187* for details.
Sensorgrams	Displays sensorgrams for the interactions selected in the ***Heat map*** or ***Table*** panels. Boundary lines for the response read-off area (vertical lines) and blocking/non-blocking interactions (horizontal lines) are shown. See *Section 8.2.3 Sensorgrams panel, on page 189* for details.
Heat map	Displays the interaction type for each pair of antibodies in a color coded matrix. Selected cells are highlighted and corresponding interactions are shown in the ***Sensorgrams*** panel and are highlighted in the ***Table*** panel. See *Section 8.2.4 Heat map panel, on page 193* for details.
Bin chart	Displays how all the antibodies included in the ***Epitope binning*** item are grouped in different bins and bin clusters. See *Section 8.2.5 Bin chart panel, on page 197* for details.

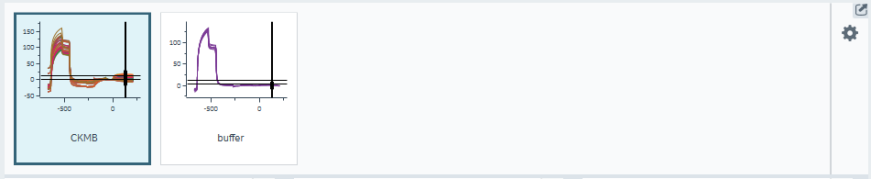
Panel	Content
Table	Displays data for all sensorgrams in the evaluation. Selected rows are highlighted and corresponding interactions are shown in the ***Sensorgrams*** panel and are highlighted in the and ***Heat map*** panel. See *Section 8.2.6 Table panel, on page 200* for details.

Use the buttons at the bottom of the ***Settings*** panel to show or hide selected panels. The panels are described in more detail in the following subsections.

8.2.2 Thumbnails panel

Panel description

The ***Thumbnails*** panel displays thumbnails of the data grouped according to the ***Data grouping*** setting. The thumbnails panel is hidden by default, as the default data grouping setting for epitope binning often results in only one group.

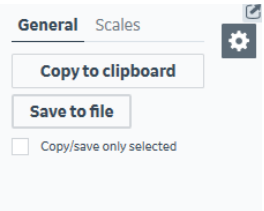


Select a thumbnail to select data group for evaluation. Selected thumbnails are marked by a heavy dark blue border. Each group is evaluated individually.

Panel toolbar

Click ***Thumbnail settings*** on the panel toolbar to access display settings for the panel. Settings are applied to all thumbnails. The settings are divided into two sections, ***General*** and ***Scale***.

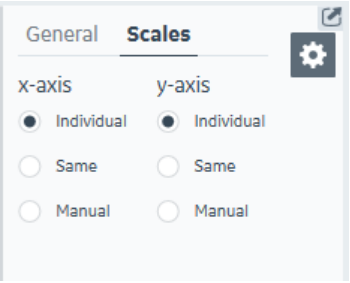
General tab



Setting	Description
Copy to clipboard	Copies thumbnails to the Windows clipboard as a collection of graphical objects. The thumbnails can be pasted into programs that support pasting for collections of graphical objects. Check ***Copy/save only selected*** to copy only selected thumbnails. If this option is not checked, all thumbnails will be copied.

Setting	Description
Save to file	Saves thumbnails as illustrations in ***.png*** (Portable Network Graphics) format. Each thumbnail is saved to a separate file. Check ***Copy/save only selected*** to save only selected thumbnails. If this option is not checked, all thumbnails will be saved.

Scales tab

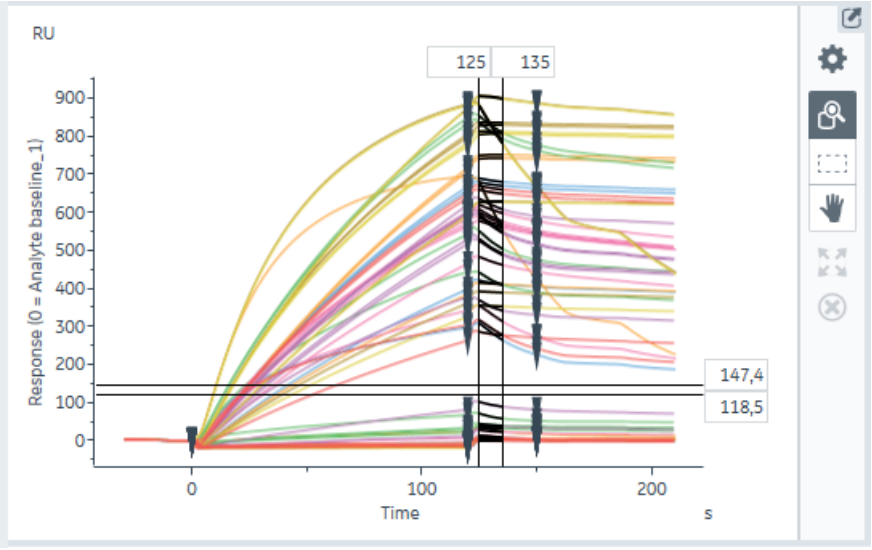


Setting	Description
x-axis ***y-axis***	Determines how the axes will be scaled in the thumbnails. • Individual scales each thumbnail separately. The scale is determined by the range of values in the thumbnail. • Same scales all thumbnails to the same scale, determined by the range of values in all thumbnails together. • Manual scales all thumbnails according to the minimum and maximum values specified.

8.2.3 Sensorgrams panel

Panel description

The ***Sensorgrams*** panel displays sensorgrams for the interactions selected in the heat map or table panel. Click on a sensorgram to select/deselect it, or use the ***Area select*** mode to select multiple sensorgrams. Selected sensorgrams are highlighted with a heavy blue line. Boundary lines for the response read-off (vertical lines) and blocking/non-blocking cut-offs (horizontal lines) are shown. Sensorgrams having their read-off below the lower cut-off line will be classified as blocking. Those above the upper cut-off will be classified as non-blocking and those in between the cut-off lines will be classified as uncertain. The position of a boundary line can be changed either by dragging the line itself or by changing the value in the box. If the position of a boundary line is changed, the other panels in the ***Epitope binning*** item will update accordingly.

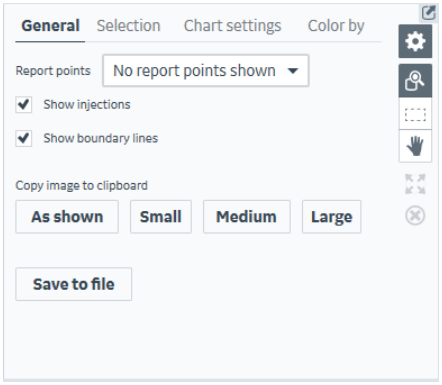


Panel toolbar

Click **Chart settings** on the panel toolbar to access display settings for the panel.

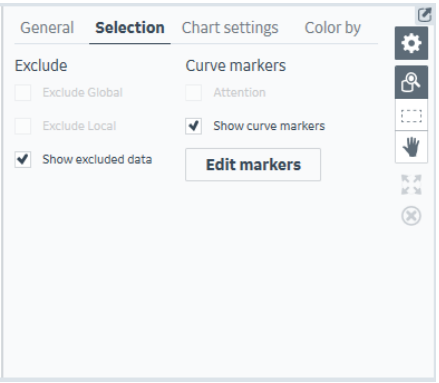
The separate tabs in the chart settings are described below. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.

General tab



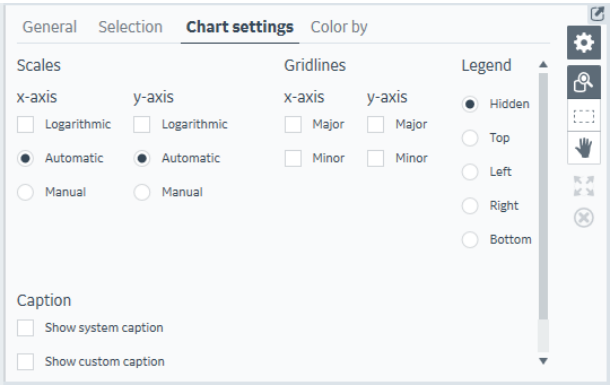
Setting	Description
Report points	Choose whether to hide report points or show markers or markers and labels.
Show injections	Check this option to show markers for the start and end of injections, including (where appropriate) the end of dissociation time.
Show boundary lines	Check this option to show boundaries for the response read-off range (vertical lines) and boundaries for blocking and non-blocking cut-offs.
Copy image to clipboard	Copies the panel as currently displayed to the Windows clipboard. Choose one of three sizes or ***As shown***. ***Note:*** ***Small***, ***Medium*** *and* ***Large*** *copies use default image proportions.* ***As shown*** *retains the proportions as displayed on the screen.*
Save to file	Saves the panel as an illustration in selectable format.

Selection tab



Setting	Description
Exclude global	Check this option to exclude the selected data from all evaluation items, including those already created. Excluding data removes any sensorgram adjustments and/or table calculations that may have been applied.
Exclude local	Check this option to exclude the selected data from the current evaluation item only.
Show excluded data	Check this option to show excluded data. Excluded points and sensorgrams are shown in gray.
Curve markers	Check the required markers to apply curve markers to selected data.
Show curve markers	Check this option to display curve markers in the sensorgram, heat map and bin chart panels.
Edit markers	Choose this option to manage curve markers.

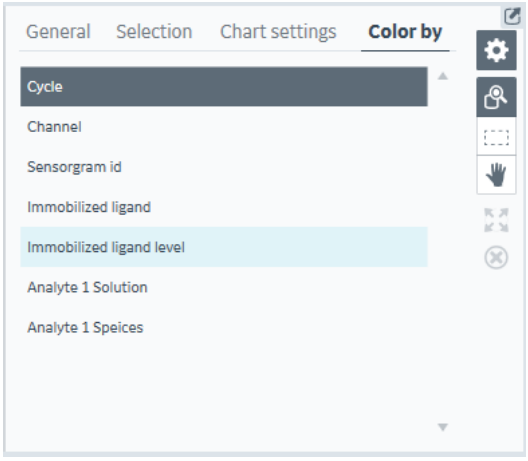
Chart settings tab



Setting	Description
Scales	Choose ***Automatic*** or ***Manual*** scales for the axes. For ***Manual*** scaling, enter minimum and maximum values. Choose ***Logarithmic*** to use a logarithmic axis scale. ***Note:*** *Zero or negative values cannot be displayed on a logarithmic scale.*
Gridlines	Choose whether to show major and/or minor gridlines. Gridline spacing is determined according to the scale range.
Legend	Displays a legend identifying sensorgrams and plot points by color. Choose the position of the legend in the panel.
Caption	Displays a caption in the panel. Choose whether to use the system caption and/or a custom caption with text as entered.

Color by tab

The ***Color by*** setting colors sensorgrams and points according to a selected property. Available options are determined by variable parameters in the run method and by any curve filters that are applied in the evaluation.

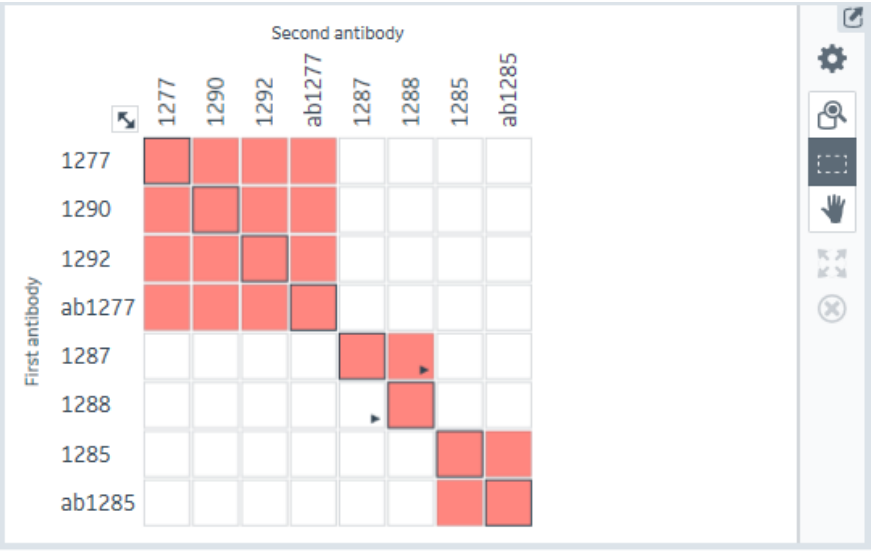


Note: *Sensorgram and point colors are hidden by the selection highlight when sensorgrams and points are selected.*

8.2.4 Heat map panel

Panel description

The ***Heat map*** panel displays the interaction type for each antibody pair in a color coded matrix. Each cell in the heat map represents the interaction between a certain first antibody (shown on the y-axis) and a certain second antibody (shown on the x-axis). By default, the heat map is automatically sorted by bin, with antibodies belonging to the same bin placed next to each other. Self-self interactions, i.e., interactions with the same antibody as both first and second antibody, are marked by a black frame.



Red cells represent blocking interactions, where the first antibody blocks the second antibody from binding to the antigen. Corresponding sensorgrams have their response read-off below the lower cut-off in the ***Sensorgrams*** panel. White cells represent non-blocking interactions, where both first and second antibodies bind simultaneously to the antigen. Corresponding sensorgrams have their response read-off above the upper cut-off in the ***Sensorgrams*** panel. Yellow cells represent uncertain interactions. These interactions might need to be inspected further, in context with other data, to assess whether they are blocking or not. Corresponding sensorgrams have their response read-off between the two cut-offs in the ***Sensorgrams*** panel. If an antibody blocks another antibody, without being blocked back by the blocked antibody, their interaction is said to be uni-directional. Uni-directional blocking is illustrated with arrows in the affected cells in the heat map.

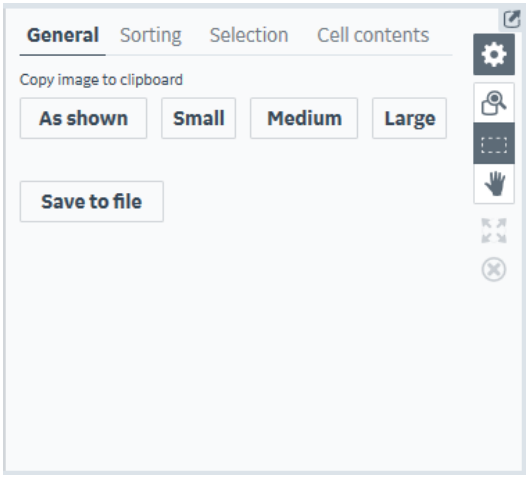
Example of a uni-directional interaction. With antibody 1287 as the first antibody, antibody 1288 is blocked from binding to the antigen. However, with antibody 1288 as the first antibody, antigen 1287 is able to bind to the antigen.

Panel toolbar

Click ***Heat map settings*** on the panel toolbar to access display settings for the panel.

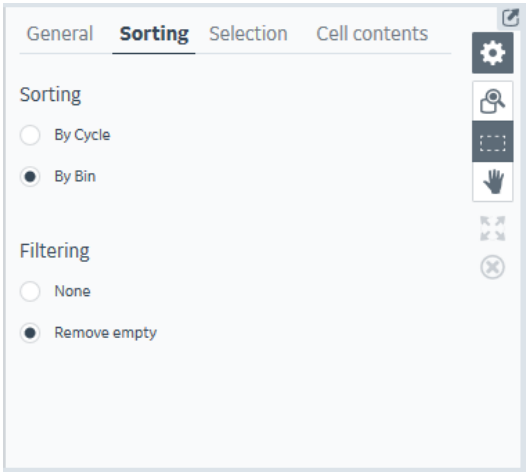
The separate tabs in the heat map settings are described below. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.

General tab



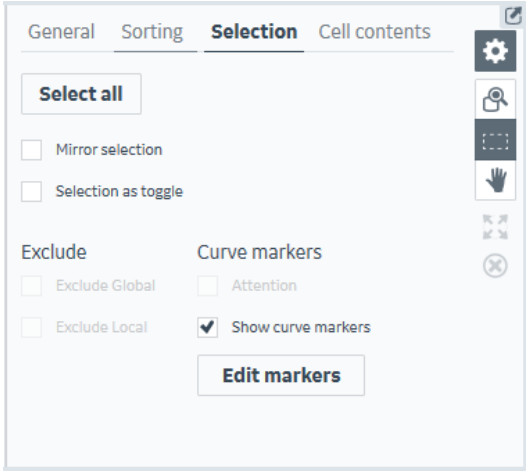
Setting	Description
Copy image to clipboard	Copies the panel as currently displayed to the Windows clipboard. Choose one of three sizes or ***As shown***. ***Note:*** ***Small***, ***Medium*** *and* ***Large*** *copies use default image proportions.* ***As shown*** *retains the proportions as displayed on the screen*
Save to file	Saves the panel as a graphics file.

Sorting tab



Setting	Description
Sorting	Choose whether to sort the results by cycle or by bin.
Filtering	Choose whether or not to remove cells in the heat map that lack experimental data.

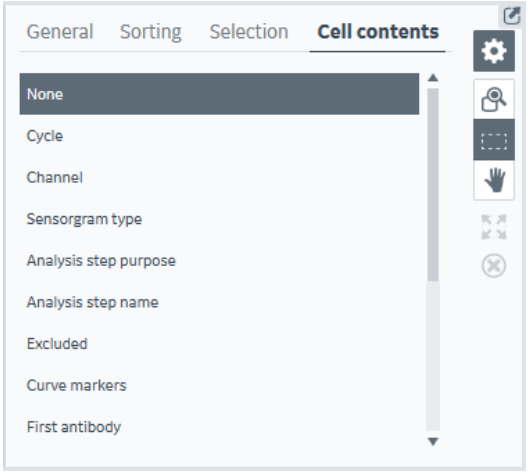
Selection tab



Setting	Description
Select all	Selects all cells in the heat map.

Setting	Description
Mirror selection	If this option is checked, clicking on a cell will select the cell **and** its mirrored counterpart, where the antibodies were injected in opposite order.
Selection as toggle	If this option is checked, click on a cell to toggle between selected and deselected status. When the option is not selected, clicking will only select the cell.
Exclude Global	Check this option to exclude the selected data from all evaluation items, including those already created. Excluding data removes any sensorgram adjustments and/or table calculations that may have been applied.
Exclude Local	Check this option to exclude the selected data from the current evaluation item only.
Curve markers	Check the required markers to apply curve markers to the data.
Show curve markers	Check this option to display curve markers in the sensorgram, heat map and bin chart panels.
Edit markers	Click ***Edit markers*** to open the ***Curve markers*** tool, to add, edit, or remove curve markers.

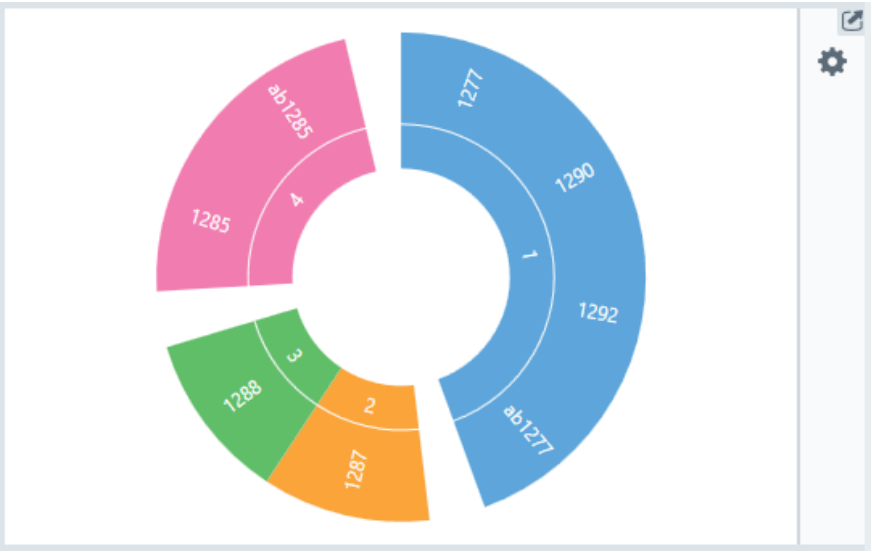
Cell contents tab



Select a column from the table to display its contents in the cells of the heat map.

8.2.5 Bin chart panel

Panel description



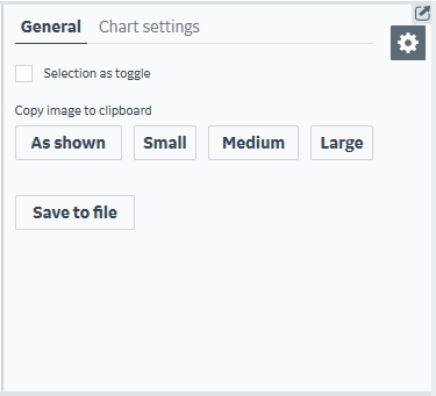
The ***Bin chart*** panel shows how all the antibodies included in the ***Epitope binning*** item are grouped in different bins. A bin is shown as a sector with common color and bin number. All antibodies within a bin share the same blocking pattern, as defined by the binning settings. Individual bins are separated by white gaps. If several bins have partially overlapping blocking patterns, they form a bin cluster and are shown directly next to each other, without any intermediate gaps. Only antibodies from separated bins can be assumed to bind to independent epitopes on the antigen.

Panel toolbar

Click ⚙ ***Chart settings*** on the panel toolbar to access display settings for the panel.

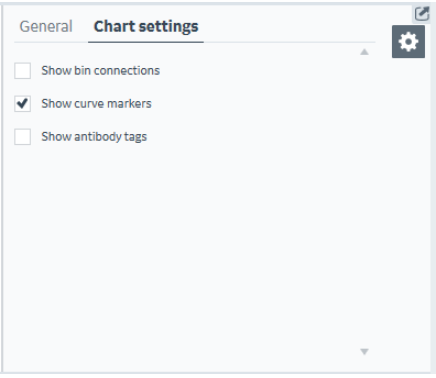
The separate tabs in the chart settings are described below. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.

General tab



Setting	Description
Selection as toggle	If this option is checked, clicking on a bin connection will toggle between selected and deselected. When the option is not selected, clicking will only select the connection.
Copy image to clipboard	Copies the panel as currently displayed to the Windows clipboard. Choose one of three sizes or ***As shown***. ***Note:*** ***Small***, ***Medium*** *and* ***Large*** *copies use default image proportions.* ***As shown*** *retains the proportions as displayed on the screen.*
Save to file	Saves the panel as an illustration in selectable format.

Chart settings

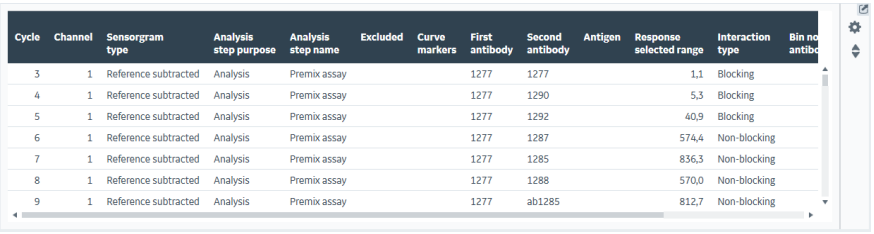


Setting	Description
Show bin connections	Check this option to display connections between bins in a cluster, that have a partially overlapping blocking pattern. Uni-directional blocking is indicated with an arrow in the blocking direction. Uncertain blocking is indicated by a dashed line.
Show curve markers	Check this option to display curve markers in the sensorgram, heat map and bin chart panels.
Show antibody tags	Check this option to display tags that hold extra information about the antibodies. Any ***Table*** column can be used as the source of information. Select which table column to use under ***Tag by***. Select whether the information in the column applies to the first or second antibody under ***Apply tag to***. Select position of the legend under ***Legend***.

8.2.6 Table panel

Panel description

The table panel lists parameter values for all sensorgrams included in the epitope binning item.



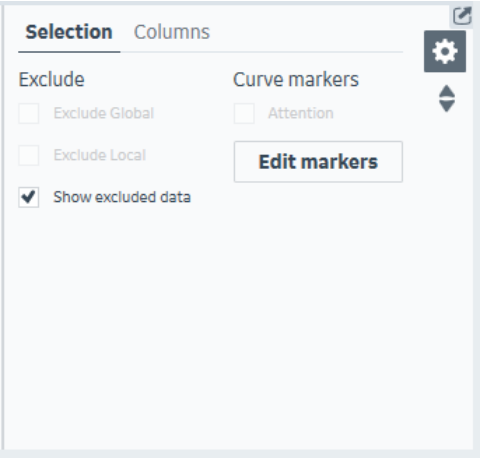
Cycle	Channel	Sensorgram type	Analysis step purpose	Analysis step name	Excluded	Curve markers	First antibody	Second antibody	Antigen	Response selected range	Interaction type	Bin no antibo
3	1	Reference subtracted	Analysis	Premix assay			1277	1277		1,1	Blocking	
4	1	Reference subtracted	Analysis	Premix assay			1277	1290		5,3	Blocking	
5	1	Reference subtracted	Analysis	Premix assay			1277	1292		40,9	Blocking	
6	1	Reference subtracted	Analysis	Premix assay			1277	1287		574,4	Non-blocking	
7	1	Reference subtracted	Analysis	Premix assay			1277	1285		836,3	Non-blocking	
8	1	Reference subtracted	Analysis	Premix assay			1277	1288		570,0	Non-blocking	
9	1	Reference subtracted	Analysis	Premix assay			1277	ab1285		812,7	Non-blocking	

Panel toolbar

Click ***Table settings*** on the panel toolbar to access display settings for the panel.

The separate tabs in the table settings are described below. ***Sort on selection*** groups selected rows at the top or bottom of the table. Click repeatedly to switch between top and bottom of the table.

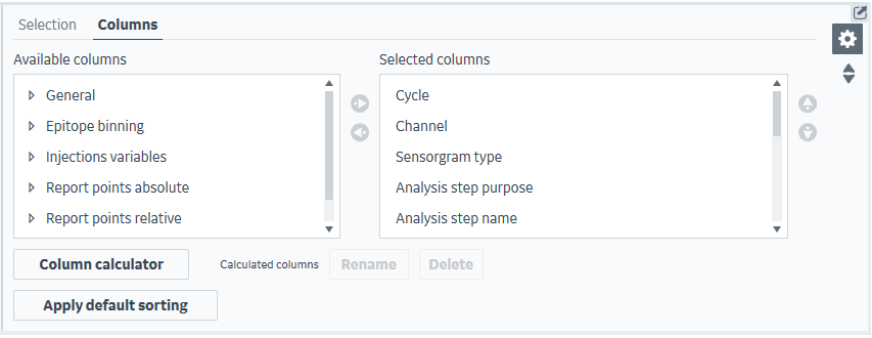
Selection tab



Setting	Description
Exclude global	Check this option to exclude the selected data from all evaluation items, including those already created. Excluding data removes any sensorgram adjustments and/or table calculations that may have been applied.

Setting	Description
Exclude local	Check this option to exclude the selected data from the current evaluation item only.
Show excluded data	Check this option to show excluded data in the sensorgram panel. Excluded sensorgrams are shown as a gray line.
Curve markers	Check the required markers to apply curve markers to the selected data.
Edit markers	Choose this option to manage curve markers.

Columns tab



Move columns between ***Available columns*** and ***Selected columns*** to change the information displayed in the table. Select a column in the ***Selected columns*** list and use the up and down arrows to change the column display order.

Select ***Column calculator*** to define table columns that hold results calculated from other column values. See *Section 6.8 Calculated columns, on page 148* for details.

Click ***Apply default sorting*** to restore the default sort order for table rows if you have changed the sort order.

Note: *Applying default sorting does not affect the column order.*

8.3 Epitope binning item settings

Introduction

This section describes the available settings in the ***Settings*** panel at the left of the plot workspace. The ***Select sensorgrams*** panel is described in *Workspace settings, on page 30*.

Data grouping

The ***Data grouping*** setting determines how the data is divided into groups. The number of groups is shown in parentheses in the ***Data grouping*** setting header.

The following ***Data grouping*** settings are available:

Setting	Description
Default	Default setting by the software. All cycles with the same antigen solution and command settings will be grouped together.
All together	Plots all the data in a single group.

Injection assignment

The ***Injection assignment*** setting determines which injection command that was used for the first antibody, the antigen, and the second antibody, respectively. Use ***Capture (multiple)*** for 1-channel data if different antibodies or antigens were captured in different flow cells in the same cycle. The first antibody and the antigen can also be assigned to ***Immobilized ligand***. Information from the specified injections will be used by the heat map, bin chart, table and the binning algorithm.

Note: *In situations where antigen and second antibody have been mixed outside the instrument and injected using the same injection command, select the appropriate injection for the second antibody and set the antigen injection to* ***None****.*

Alignment

The ***Alignment*** settings determine how the sensorgrams are aligned to zero response and time. The settings are applied to all sensorgrams in the evaluation, and affect the graphical display in the sensorgram and thumbnail panels.

Choose whether to align to an injection event or a report point. Choose ***No alignment*** to display actual response values. The setting is independent for x- and y-axes.

Sensorgrams are aligned by default to the baseline report point just before the second antibody injection, with zero on the x-axis at the beginning of the injection and zero on the y-axis at the baseline report point.

Dual injections are aligned by default with zero on the x-axis at the beginning of the Dual B part of the injection and zero on the y-axis at the Dual A binding late report point. The corresponding positions for Poly injections are the beginning of the Poly C part, and the Poly B binding late report point for x-axis respectively y-axis alignment.

Select ***Use normalization*** and choose whether to normalize to a report point or an injection event. Select which report point or injection event that sets the low value of the normalization interval. Select which report point or injection event that sets the high value of the normalization interval. In the graph, the low value is not necessarily zero, and the high value not necessarily 100, as that is determined by the alignment settings. However, the sensorgrams are mathematically normalized according to zero for the low point and 100 for the high point.

Sensorgram subtraction

The ***Sensorgram subtraction*** setting allows sensorgrams from one cycle to be subtracted from all others. Subtraction is performed within flow cells (1-channel data) or channels (8-channel data).

Subtract sef-self sensorgram subtracts the response of sensorgrams that have the same first and second antibody from all sensorgrams that have that antibody as the first antibody.

Subtract blank subtracts the response in blank sensorgrams from all sensorgrams in the same flow cell (1-channel data) or channel (8-channel data), according to the sample blank pairing set by the sequence selection of ***Preceding*** blank, ***Following*** blank, ***Nearest*** blank or ***Median of nearest*** blanks. Select which injection command that holds the blank. Blank sensorgrams may be sample cycles with solutions defined as ***Blank*** or with zero concentration, or negative control cycles.

Subtract cycle subtracts the sensorgrams for a specified cycle from all other others in the same flow cell (1-channel data) or channel (8-channel data). Subtraction is not performed if the sensorgrams do not share a common alignment point (for example, solvent correction cycles will not be subtracted from analyte injection cycles).

Binning settings

The ***Bin definition*** allows uni-directional blocking (antibody pairs that only block in one order of first and second antibody) to be included in the bins.

Uncertain responses, that fall in between the blocking and non-blocking cut-offs, can be treated as follows:

Setting	Description
Separately	Uncertain responses are grouped in separate bins.
As blocking	Uncertain responses are grouped in bins as if they were blocking.
As non-blocking	Uncertain responses are grouped in bins as if they were non-blocking.

Antibody names

The ***Antibody names*** setting allows numbers to be shown instead of antibody names. This setting is recommended if the antibody names are long.

8.4 Workflow for epitope binning evaluation

Procedure

A recommended workflow for evaluating epitope binning experiments is given below.

Step	Action
1	Create an ***Epitope binning*** item.
2	Verify that the ***Data grouping*** setting correlates with how the run was set up, adjust if needed. • When ***Default*** is active, cycles with the same antigen solution and command settings are grouped together. • When ***All together*** is active, all the data is in a single group.
3	Verify that the auto populated ***Injection assignment*** settings reflect the injection sequence in the runs, adjust if needed.
4	Verify that the auto populated ***Alignment*** settings suits the evaluation purposes, both ***x-axis*** and ***y-axis*** settings, normalization is selected later. Sensorgrams are aligned per default to the baseline report point directly before the second antibody injection.
5	Exclude any disturbed cycles from the evaluation.
6	If blank injections were included in the run, examine the sensorgrams to assess whether there are drift or systematic disturbances that can be removed by blank subtraction. If so, open the ***Sensorgram subtraction*** settings and perform blank subtraction. Then, remove the blank cycles from the evaluation by unchecking them in the ***Select sensorgrams*** settings.
7	If there are antibodies that have consistently low antigen binding response levels compared to the general level of the run, consider to remove the antibodies from the evaluation by unchecking them in the ***Select sensorgrams*** settings.
8	If the antibody capture or antigen binding response levels vary considerably between different first antibodies, select ***Use normalization*** in the ***Alignment*** settings. If the variation is large, the normalization reduces the effect of irrelevant variations on the response levels for the second antibodies.
9	If needed, in the ***Sensorgrams*** panel, adjust the response read-off range by dragging the vertical lines or by entering numbers in the boxes.

Step	Action
10	In the ***Heat map*** panel, select all cells with the same antibody as both first and second antibody, to use their sensorgrams as controls of blocking interactions. Examine them in the ***Sensorgrams*** panel and adjust the lower cut-off by dragging the horizontal line, or by entering numbers in the box. ***Tip:*** *Clicking*  *in the upper left corner select all cells with the same antibody as both first and second antibody.*
11	In the ***Heat map*** panel, select all yellow cells (indicating uncertain interaction, i.e., between the two cut-offs), alternatively, in the ***Table*** panel, select all rows that have ***Interaction type*** > ***Uncertain***. Examine them in the ***Sensorgrams*** panel and adjust the upper cut-off, and the lower cut-off again if appropriate. Select some white and red cells as reference.
12	Examine the results in the ***Bin chart***. If there are uni-directional or uncertain interactions in the evaluation, click on ***Binning settings*** to select how those interactions are treated by the binning algorithm.
13	If there are clustered bins in the ***Bin chart***, select ***Chart settings*** > ***Show bin connections*** to display how the bins are connected. Separate bins are shown with gaps to adjacent bins, while clustered bins that share a partially overlapping blocking pattern are kept together.

9 Kinetics and Affinity items

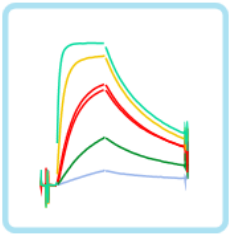
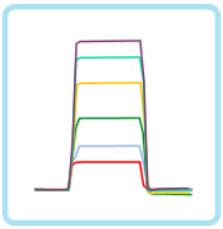
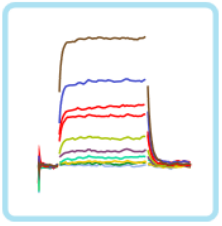
About this chapter

This chapter describes how to use ***Kinetics***, ***Affinity***, and ***Kinetics and affinity*** items. They are three modes of the same item, varying in settings and what information they provide. It is possible to change the mode within the item.

In this chapter

Introduction

Kinetics and affinity items are used for obtaining kinetic constants (association rate constants k_a and dissociation rate constants k_d) and/or affinity constants (K_D) by fitting mathematical interaction models to the experimental data. The appearance of the sensorgrams determines whether kinetic or affinity information (or both) can be obtained from an experiment, as detailed in the table below. In some cases, it may be possible to obtain kinetic information for dissociation but not for association.

Information	Sensorgram appearance	Example
Kinetics only	Sensorgrams show curvature during association and dissociation, but do not reach steady state during association.	
Affinity only	Sensorgrams reach steady state during association, but association and dissociation are too rapid to show sufficient curvature for kinetic evaluation.	
Kinetics and affinity	Sensorgrams reach steady state during association ***and*** show sufficient curvature for kinetic evaluation.	

More background and details for kinetic and affinity evaluation principles are found in *Appendix A Curve fitting procedures, on page 286*.

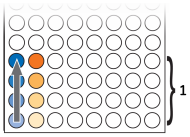
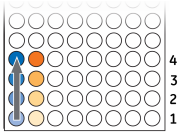
9.1 Experimental formats

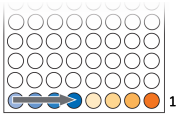
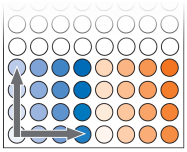
Analysis modes and microplate layout

Biacore Insight Evaluation software supports evaluation of several approaches to determination of kinetics and affinity:

- In ***single-cycle*** mode, analyte concentrations are injected in series in a single cycle, with no regeneration between injections. Regeneration may be performed after the last analyte injection to prepare the surface for a new analyte. The ligand, analyte or both can be varied between channels in 8-channel systems, while only the ligand can be varied between flow cells in 1-channel systems.
- In ***multi-cycle*** mode, each analyte concentration is injected in a separate cycle, and the surface is regenerated between cycles. The ligand, analyte or both can be varied between channels in 8-channel systems, while only the ligand can be varied between flow cells in 1-channel systems..
- In ***parallel*** mode, each analyte concentration is injected in the same cycle but in a different channel. The same ligand is immobilized in the different channels.
- ***2D*** mode is a combination of single-cycle and parallel modes, allowing a wide analyte concentration range to be tested in a single experiment.

Parallel and 2D modes are only supported by 8-channel instruments such as Biacore 8K and Biacore 8K*. The table below illustrates the microplate layout for the different analysis modes for 2 analyte determinations without blank cycles. Remember that blank cycles must be run in the same flow cells (1-channel data) or channels (8-channel data) as the analyte. The numbers on the right of the illustrations indicate numbers of cycles.

Mode	Description	Illustration
Single-cycle	All concentrations are injected in series in the same cycle. For 8-channel systems, the concentration series for one analyte is distributed along a column in the microplate. Four analyte concentrations occupy four rows. 1-channel systems offer alternative positioning options.	
Multi-cycle (serial mode)	Each concentration is injected in a separate cycle. For 8-channel systems, the concentration series for one analyte is distributed along a column in the microplate. Four analyte concentrations occupy four rows. 1-channel systems offer alternative positioning options.	

Mode	Description	Illustration
Parallel	The concentration series for one analyte is distributed across a row in the microplate. Since 8 positions on a row are always used in parallel, one cycle can inject a series of 4 concentrations for 2 analytes.	
2D	The concentration series for one analyte is distributed along a column in the microplate as in the single-cycle arrangement. Different concentration series of the same analyte are analyzed in each channel to cover a wider range of concentrations.	

9.2 Requirements for kinetics and affinity analysis

Minimum requirements

The minimum requirements for evaluation of kinetics or affinity are one cycle with an ***Analyte*** injection in an assay step with purpose ***Analysis***, and with the sample concentration in the ***Concentration*** variable. If the concentration is not given in molar-based units, the ***Molecular weight*** variable must also be included with a value for the molecular weight. If necessary, the variables can be edited after the run is completed to meet the conditions (see *Section 3.3 Variables, on page 40*). Note however that the injection type cannot be edited in the keyword table.

Evaluation of steady state affinity requires at least 3 non-zero analyte concentrations.

To see the requirements for affinity screens when using ***Biacore Intelligent Analysis***, see *Section 9.6.2 Requirements on affinity screen runs, on page 251*.

Recommendations

The recommended minimum conditions for detailed kinetic and affinity analysis are:

- a concentration series of analyte with at least three non-zero concentrations
- at least one blank cycle consisting of zero concentration sample (for single-cycle kinetics the blank cycle must replicate the sequence of injections in the analysis cycle)
- for multi-cycle kinetics, duplicate determinations for at least one non-zero concentration

Kinetic screening (to estimate approximate values for kinetic constants) can be performed at a single analyte concentration. Affinity screening, like detailed affinity analysis, requires at least 3 (recommended 4) analyte concentrations to provide sufficient data for evaluation. Blank cycles are recommended, although the same cycle may be used as a blank for several different analytes.

9.3 Workflow for kinetics and affinity evaluation

Procedure

A recommended workflow for evaluating kinetics and affinity experiments is given below.

Step	Action
1	Based on previous knowledge or expectations of the interaction properties, create an evaluation item for ***Kinetics***, ***Affinity***, or ***Kinetics and affinity***. If you are unsure which to create, use ***Kinetics and affinity***. The mode can be changed if necessary for selected data series in the item.
2	For data from 8-channel systems, choose the ***Data grouping*** setting according to how the run was set up (see *Data grouping, on page 242*).
3	Verify that the ***Injection assignment*** is appropriate, change if needed (see *Injection assignment, on page 242*).
4	Examine the thumbnails for evaluation potential. Switch between ***Sensorgrams*** and ***Plots*** in ***Thumbnail settings*** for different views of the data. **a.** Reject any series that are not appropriate for evaluation (for example, where the data is disturbed). **b.** Select series that show kinetic data but do not reach steady state, and set the ***Kinetics/Affinity mode*** to ***Kinetics***. **c.** Select series that reach steady state but do not show kinetic data, and set the ***Kinetics/Affinity mode*** to ***Affinity***. **d.** Select series that reach steady state and also show kinetic data, and set the ***Kinetics/Affinity mode*** to ***Both***.
5	Set the required blank subtraction mode (see *Blank settings, on page 243*). The default mode is suitable for most purposes if you have included blank (zero-concentration) cycles for all concentration series in the run. Examine the blank cycles for each series and exclude any disturbed cycles.
6	Choose appropriate fitting models for all series to be evaluated (see *Fit models, on page 244*).
7	Click ***Perform fit***. The number in parentheses on this button indicates the number of data series for which fitting will be performed.
8	Review the results. Set the thumbnail selection status to ***Accepted*** for approved results. This will prevent refitting the series with new settings or model choices.

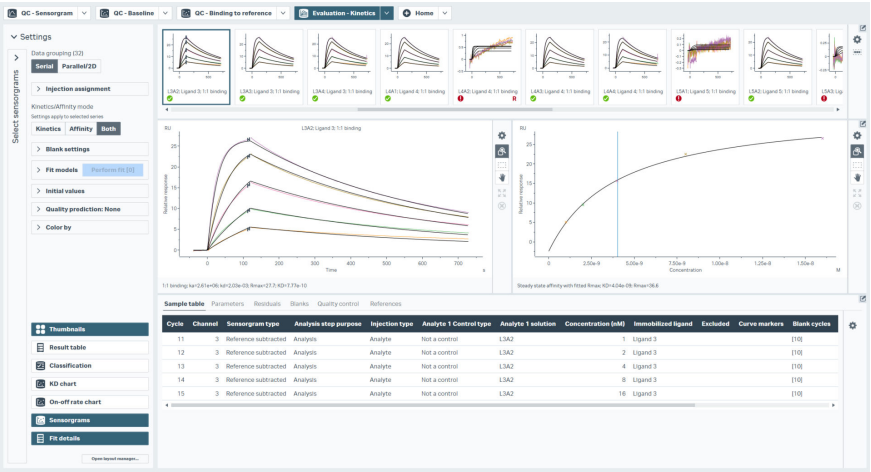
Step	Action
9	Change settings and refit selected series as required. Changing settings for a series will delete any previous fitting data for that series.

9.4 The Kinetics and Affinity workspaces

In this section

9.4.1 Introduction

The ***Kinetics and affinity*** item workspace holds up to seven panels in addition to the ***Settings*** panel at the left of the workspace:



Content	Content
Thumbnails panel	Displays the data in the evaluation grouped in sample concentration series. In ***Kinetic*** and ***Both*** modes, the thumbnails show the sensorgrams. In ***Affinity*** mode, the thumbnails show the steady state response plotted against concentration (R_{eq} versus C). See *Section 9.4.2 Thumbnails panel, on page 217* for more information.
Result table panel (not shown)	Displays a table with the results of completed evaluations for the last selected concentration series. See *Section 9.4.3 Result table panel, on page 222* for more information.
Classifications panel (not shown)	Displays available classifications and acceptance states. See *Classification panel, on page 257* for more information. It is visible when the ***Biacore Intelligent Analysis*** extension is active, and accessible when ***Quality prediction*** is active (set to ***Use prediction***).
KD chart panel (not shown)	Displays affinity constants (equilibrium dissociation constants, K_D) against the data series (numbered from left to right in the thumbnail order) for all completed affinity evaluations. See *Section 9.4.5 KD chart panel, on page 226* for more information.

Content	Content
On-off rate chart panel (not shown)	Displays a plot of association rate constants (on rate, k_a) against dissociation rate constants (off rate, k_d) for all completed kinetic evaluations using the 1:1 binding model. See *Section 9.4.6 On-off rate chart panel, on page 229* for more information.
Sensorgrams panel	In ***Kinetic*** mode, the ***Sensorgrams*** panel displays the sensorgrams for the most recently selected thumbnail. In ***Affinity*** and ***Both*** modes, the ***Sensorgrams*** panel is divided into two sub-panels, showing the sensorgrams on the left and the steady state response plotted against concentration (R_{eq} versus C) on the right. See *Section 9.4.7 Sensorgrams panel, on page 232* for more information.
Fit details panel	Displays the details for the currently selected series. See *Section 9.4.8 Fit details panel, on page 238* for more information.

Drag the panel borders to resize the panels.

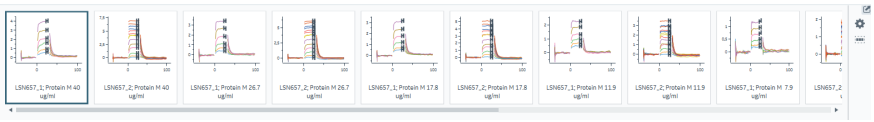
Use the buttons at the bottom of the ***Settings*** panel to show or hide selected panels.

The panels are described in more detail in the following subsections.

9.4.2 Thumbnails panel

Panel description

The ***Thumbnails*** panel displays thumbnails of the data series based on interaction characteristics (analyte, ligand and injection conditions) and according to flow cell (1-channel data) or the ***Data grouping*** setting (8-channel data). Default settings show sensorgrams for ***Kinetics*** items and steady state response data plotted against sample concentration data for ***Affinity*** items. You may need to scroll the panel display to see all thumbnails.



Select one or more thumbnails to select data series for evaluation. Use **Shift-click** and **Ctrl-click** to select multiple thumbnails. Selected thumbnails are marked by a heavy light or dark blue border. Only the series with a heavy dark blue border (the most recently clicked thumbnail) is shown in the detail panels. All selected thumbnails will be evaluated.

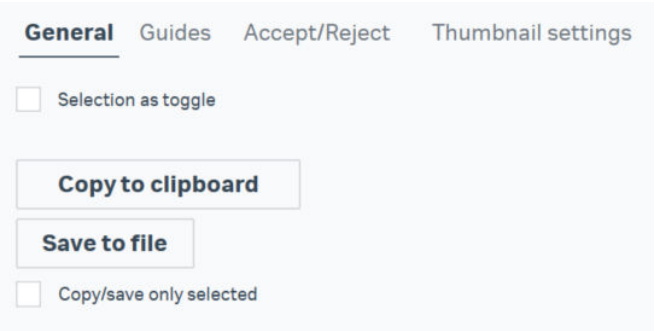
Tip: *To browse through the selected thumbnails without changing the selection, choose* ***Selection as toggle*** *in the* ***General*** *tab under* ***Thumbnail settings*** *and then click twice on a thumbnail with a light blue border. The first click will deselect the thumbnail, the second will select it with a heavier black border. Using* **Ctrl-click** *has the same effect regardless of the* ***Selection as toggle*** *status.*

Deselect ***Thumbnails*** in the ***Settings*** panel at the left to collapse the thumbnails to buttons. Data groups can be selected with these buttons even when the thumbnail content is not displayed.

Panel toolbar

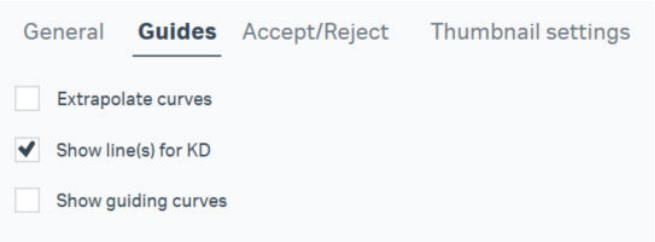
Setting	Description
Thumbnail settings	Opens the display settings for the thumbnails panel. Tabs in the display settings are described below.
Select all thumbnails	Selects all thumbnails in the thumbnails panel, and all rows in the result table panel.

General tab



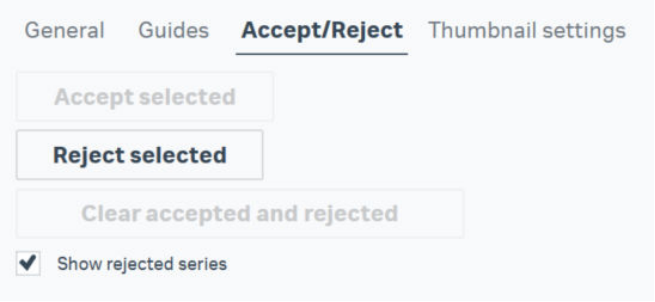
Setting	Description
Selection as toggle	If this option is checked, clicking on a thumbnail will toggle between selected and deselected. When the option is not selected, clicking will only select the thumbnail. ***Note:*** *When the option is checked, using* **Shift-click** *will toggle the status of all thumbnails in the range covered.*
Copy to clipboard	Copies thumbnails to the Windows clipboard as a collection of graphical objects. The thumbnails can be pasted into programs that support pasting for collections of graphical objects. Check ***Copy/save only selected*** to copy only selected thumbnails. If this option is not checked, all thumbnails will be copied.
Save to file	Saves thumbnails as illustrations in ***.png*** (Portable Network Graphics) format. Thumbnails are saved with the detail displayed on the screen (see *Thumbnail settings tab, on page 220*). Each thumbnail is saved to a separate file. File names are constructed from the thumbnail label with an added serial number to prevent duplicate names. Check ***Copy/save only selected*** to save only selected thumbnails. If this option is not checked, all thumbnails will be saved.

Guides tab



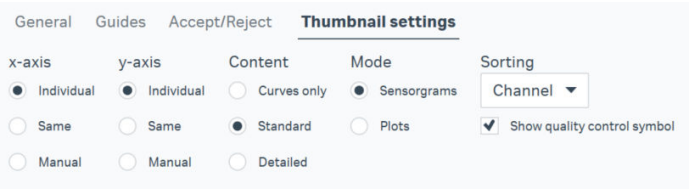
Setting	Description
Extrapolate curves	If this option is checked, the fitted dose response curves are extrapolated to a wider concentration range.
Show line(s) for KD	If this option is checked, a K_D line for each fitted curve is shown. The vertical line(s) is at the K_D concentration. The line is red if the value is considered to be outside the range for confident measurement.
Show guiding curves	If this option is checked, dose response curves for fits using fitted R_{max} and constant R_{max}, as well as a horizontal line (dashed) corresponding to the expected R_{max}, are shown.

Accept/Reject tab



Setting	Description
Accept selected	Marks selected thumbnails as ***Accepted***. Thumbnails can only be marked as ***Accepted*** after fitting has been performed. Accepted series are marked with **A** in the bottom right corner of the thumbnail. Thumbnails that are marked as ***Accepted*** cannot be re-evaluated.
Reject selected	Marks selected thumbnails as ***Rejected***. Thumbnails can be marked as ***Rejected*** before or after fitting has been performed. Rejected series are marked with **R** in the bottom right corner of the thumbnail. Thumbnails that are marked as ***Rejected*** cannot be evaluated.
Clear accepted and rejected	Clears the ***Accepted/Rejected*** status for the selected series.
Show rejected series	Check this option to show rejected thumbnails in the panel.

Thumbnail settings tab



Setting	Description
x-axis ***y-axis***	Determines how the axes will be scaled in the thumbnails. • ***Individual*** scales each thumbnail separately. The scale is determined by the range of values in the thumbnail. • ***Same*** scales all thumbnails to the same scale, determined by the range of values in all thumbnails together. • ***Manual*** scales all thumbnails according to the minimum and maximum values specified. • ***Logarithmic*** scales the thumbnails logarithmically, determined by the range of values in all thumbnails together. Logarithmic scale is only applies on the x-axis.
Content	Choose ***Curves only***, ***Standard*** or ***Detailed***. The options show different amounts of information in the thumbnail.
Mode	Choose ***Sensorgrams*** or ***Plots***. • ***Sensorgrams*** displays sensorgram data. • ***Plots*** displays steady state response against sample concentration. ***Note:*** ***Detailed*** *thumbnails (see* ***Content*** *above) for fitted concentration series show kinetic results in* ***Sensorgrams*** *mode and affinity results in* ***Plots*** *mode.*
Sorting	Choose the parameter for sorting thumbnails in the panel.
Show quality control symbol	Shows quality symbol for kinetic fits. See *Quality control tab, on page 240* for details.

9.4.3 Result table panel

Panel description

The ***Result table*** panel lists details of all data series in the evaluation, including fitting results for data series where fitting has been performed. Rows for the currently selected series are highlighted. The columns displayed in the table can be customized.

General Kinetics model	Curve markers	Channel	Injection variables Capture 1 Solution	Analyte 1 Solution	Quality Kinetics Chi² (RU²)	1:1 binding ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	tc
1:1 binding		1	Anti-Beta2micro	Beta2micro	8.94e-03	2.54e+06	2.64e-03	1.04e-09	23.4	3.19e+07
1:1 binding		2	Anti-Beta2micro	Beta2micro	6.54e-03	2.63e+06	2.61e-03	9.91e-10	26.6	3.15e+07
1:1 binding		3	Anti-Beta2micro	Beta2micro	1.23e-01	2.38e+06	2.56e-03	1.08e-09	25.7	4.19e+07
1:1 binding		4	Anti-Beta2micro	Beta2micro	7.67e-03	2.60e+06	2.59e-03	9.97e-10	29.2	3.40e+07

A field above the result table displays tabs, showing the number of data series classified as ***Accepted***, ***Rejected***, ***Uncertain*** and ***All***.

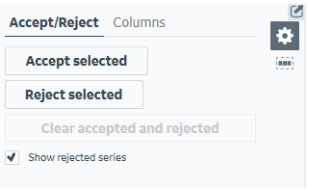
Acceptance state is either specified manually, see *Table setting options, on page 222*, or by a quality prediction, see *Section 9.6.3 Workflow for affinity screen evaluation using Biacore Intelligent Analysis evaluation method, on page 252* (requires the ***Biacore Intelligent Analysis*** extension).

These tabs function as filters. Click a category to display the corresponding thumbnails and samples in the result table.

Panel toolbar

Setting	Description
Table settings	Opens the display settings for the panel. Tabs in the display settings are described below.
Select all rows	Selects all rows in the result table and thumbnails in the thumbnails panel.

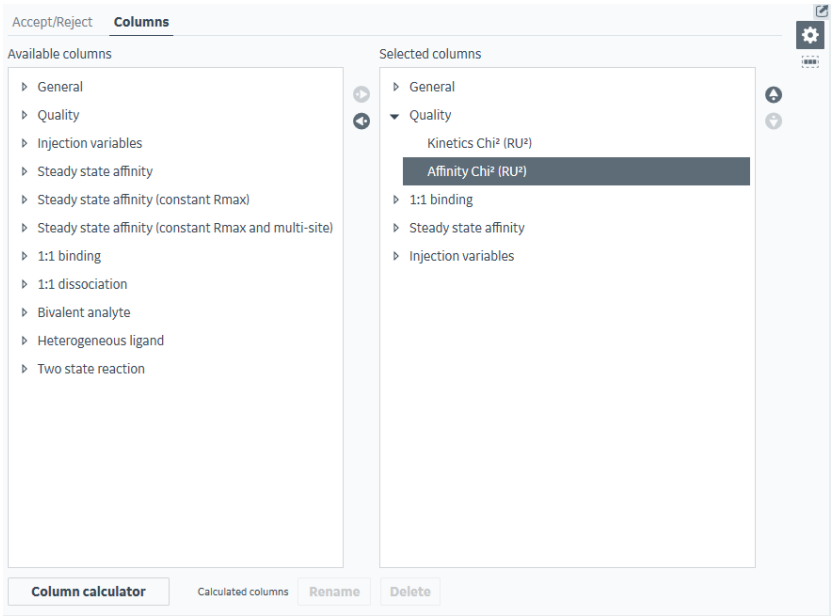
Table setting options



Setting	Description
Accept selected	Marks selected series as ***Accepted***. Series can only be marked as ***Accepted*** after fitting has been performed. Accepted series are marked with **A** in the bottom right corner of the thumbnail. Series that are marked as ***Accepted*** cannot be re-evaluated.
Reject selected	Marks selected series as ***Rejected***. Series can be marked as ***Rejected*** before or after fitting has been performed. Rejected series are marked with **R** in the bottom right corner of the thumbnail. Series that are marked as ***Rejected*** cannot be evaluated.
Clear accepted and rejected	Clears the ***Accepted/Rejected*** status for the selected series.
Show rejected series	Check this option to show rejected series in the panel.

Note: *When multiple series are selected, fitting settings and actions cannot be applied to any selected series if the last selected series is marked as* ***Accepted*** *or* ***Rejected.***

Columns tab

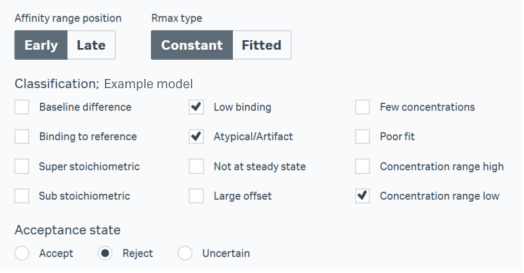


Move columns between ***Available columns*** and ***Selected columns*** using the left and right arrow buttons to change the information displayed in the table. Select a column in the ***Selected columns*** list and use the up and down arrow buttons to change the column display order.

Click ***Column calculator*** to define table columns that hold results calculated from other column values, see *Section 6.8 Calculated columns, on page 148*.

9.4.4 Classification panel

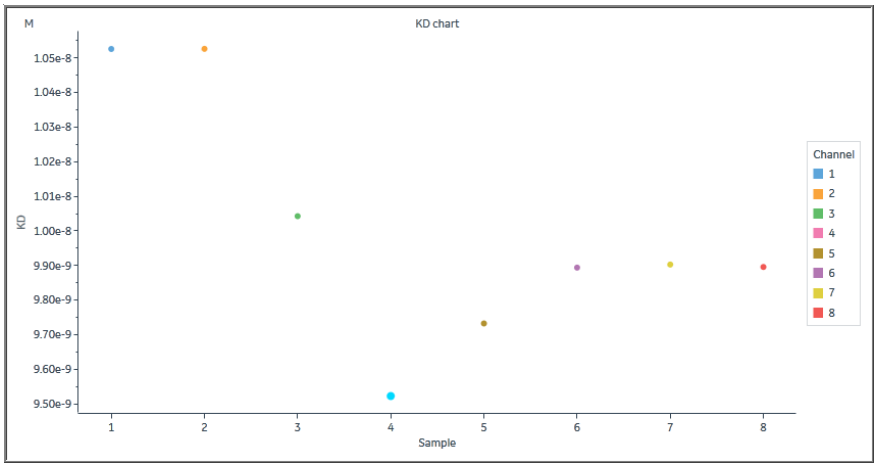
The ***Classification*** panel is available with the ***Biacore Intelligent Analysis*** extension, when a prediction has been performed. It is divided into three sections; one for defining the fit settings, one for setting classifications and one for setting the acceptance state. See *Section 9.6.3 Workflow for affinity screen evaluation using Biacore Intelligent Analysis evaluation method, on page 252* for more information.



9.4.5 K_D chart panel

Panel description

The K_D chart panel displays the steady state affinity constants (equilibrium dissociation constants, K_D) plotted against the data series (numbered from left to right in the thumbnail order) for all completed affinity evaluations.

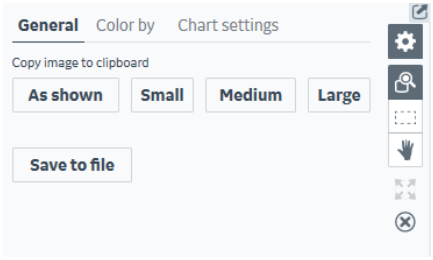


Panel toolbar

Click ***Chart settings*** on the panel toolbar to access display settings for the panel.

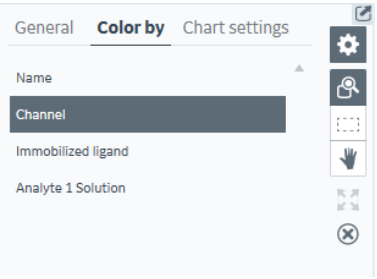
The separate tabs in the chart settings are described below. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.

General tab



Setting	Description
Copy image to clipboard	Copies the panel as currently displayed to the Windows clipboard. Choose one of three sizes or ***As shown***. ***Note:*** ***Small***, ***Medium*** *and* ***Large*** *copies use default image proportions.* ***As shown*** *retains the proportions as displayed on the screen.*
Save to file	Saves the panel as an illustration in selectable format.

Color by tab

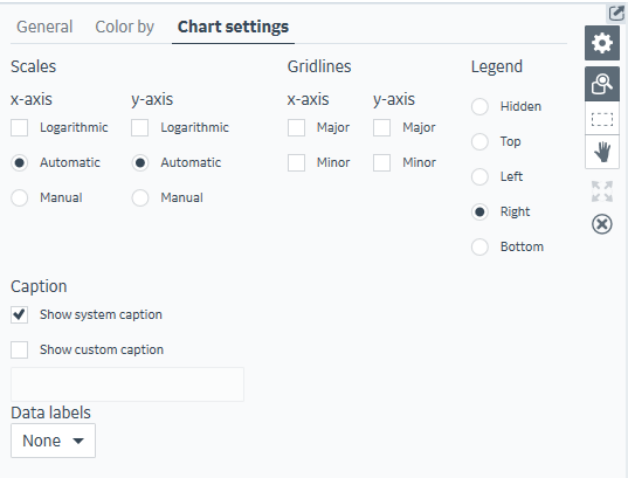


Choose the parameter by which the points will be colored. The same setting will be applied to the ***On-off rate chart*** panel.

Note: ***Color by Channel*** *is only available for 8-channel data series grouped as* ***Serial***.

Note: *Point colors are hidden by the selection highlight when points are selected.*

Chart settings tab



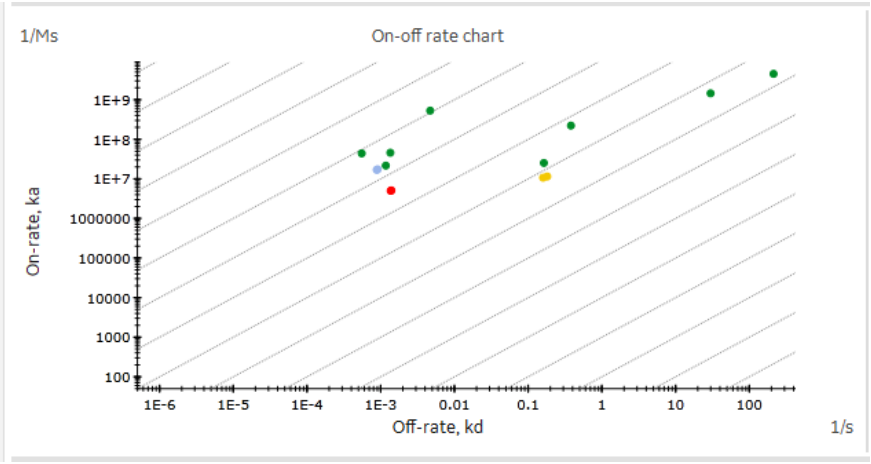
Setting	Description
Scales	Choose ***Logarithmic*** or ***Automatic*** scales for the axes. Automatic scale is always linear for sensorgram data. ***Note:*** *Zero or negative values cannot be displayed on a logarithmic scale.* Choose ***Manual*** and enter minimum and maximum values to set the scales manually.
Gridlines	Choose whether to show major and/or minor gridlines. Gridline spacing is determined according to the scale range.
Legend	Displays a legend identifying sensorgrams and plot points by color. Choose the position of the legend in the panel.
Caption	Displays a caption in the panel. Choose whether to include the system caption and/or a custom caption with text as entered.
Data labels	Displays a label for each point in the plot. Choose the property to use as label text.

9.4.6 On-off rate chart panel

Panel description

The ***On-off rate chart panel*** displays the results of kinetic evaluation as the on-rate (association rate constant k_a) plotted against the off-rate (dissociation rate constant k_d), each on a logarithmic scale by default. Points that lie on the same diagonal in a log-log plot share the same affinity constant K_D but may differ in interaction kinetics.

Points are only plotted for results of fitting to the ***1:1 binding*** model.

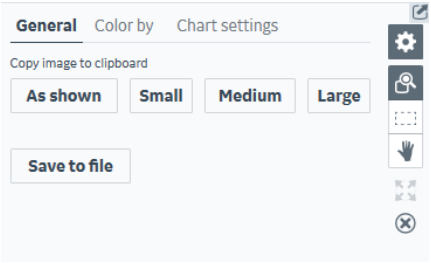


Panel toolbar

Click ⚙ ***Chart settings*** on the panel toolbar to access display settings for the panel.

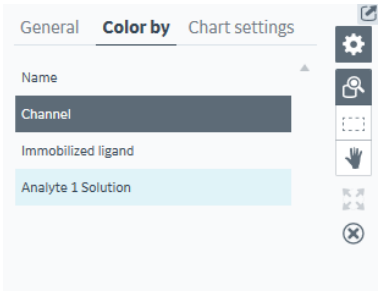
The separate tabs in the chart settings are described below. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.

General tab



Setting	Description
Copy image to clipboard	Copies the panel as currently displayed to the Windows clipboard. Choose one of three sizes or ***As shown***. ***Note:*** ***Small***, ***Medium*** *and* ***Large*** *copies use default image proportions.* ***As shown*** *retains the proportions as displayed on the screen.*
Save to file	Saves the panel as an illustration in selectable format.

Color by tab

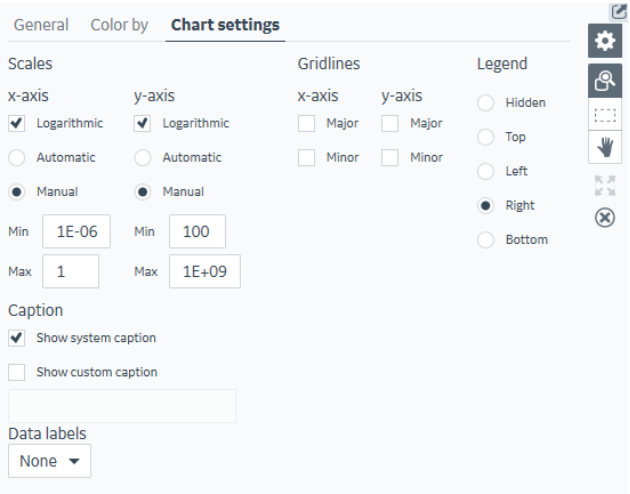


Choose the parameter by which the points will be colored. The same setting will be applied to the ***KD chart panel***.

Note: ***Color by Channel*** *is only available for 8-channel data series grouped as* ***Serial***.

Note: *Point colors are hidden by the selection highlight when points are selected.*

Chart settings tab

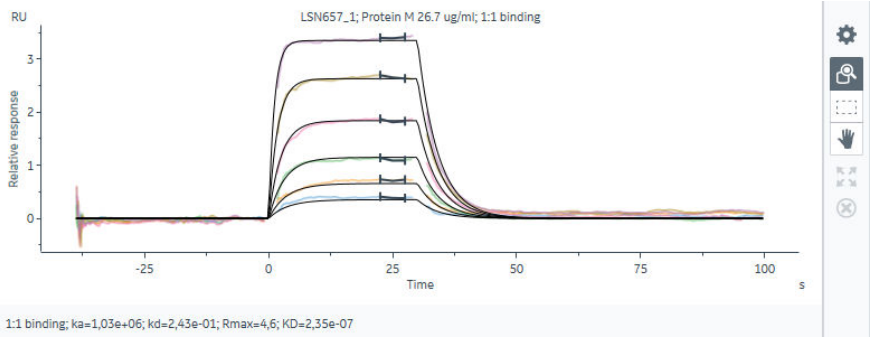


Setting	Description
Scales	Choose ***Logarithmic*** or ***Automatic*** scales for the axes. Automatic scale is always linear for sensorgram data. ***Note:*** *Zero or negative values cannot be displayed on a logarithmic scale.* Choose ***Manual*** and enter minimum and maximum values to set the scales manually.
Gridlines	Choose whether to show major and/or minor gridlines. Gridline spacing is determined according to the scale range.
Legend	Displays a legend identifying sensorgrams and plot points by color. Choose the position of the legend in the panel.
Caption	Displays a caption in the panel. Choose whether to include the system caption and/or a custom caption with text as entered.
Data labels	Displays a label for each point in the plot. Choose the property to use as label text.

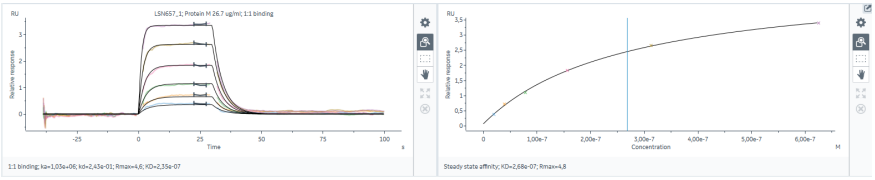
9.4.7 Sensorgrams panel

Panel description

In ***Kinetic*** mode, the ***Sensorgrams*** panel displays the sensorgrams for the most recently selected thumbnail.



In ***Affinity*** and ***Both*** modes, the ***Sensorgrams*** panel is divided into two sub-panels, showing the sensorgrams on the left and the steady state response plotted against concentration (R_{eq} versus C) on the right. The data range used to determine steady state response is marked on the sensorgrams if the option is selected in the ***Fit models*** section of the ***Settings*** panel at the left of the workspace.



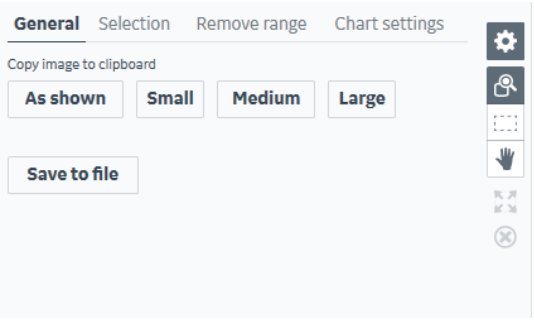
The panel displays data from only one series at a time. If multiple thumbnails are selected, the panel displays the series from the most recently selected thumbnail (marked with a heavy dark border).

Panel toolbar

Click ***Chart settings*** on the panel toolbar to access display settings for the panel. The display settings are independent for the two sub-panels.

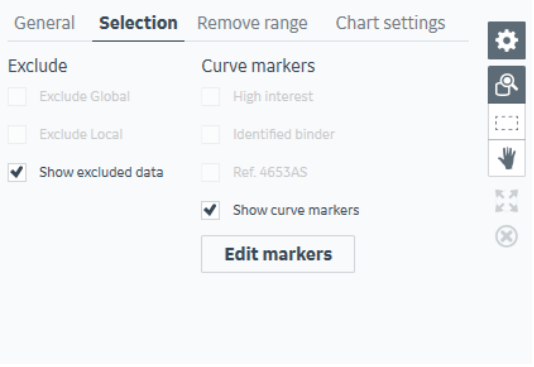
The separate tabs in the chart settings are described below. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.

General tab



Setting	Description
Copy image to clipboard	Copies the panel as currently displayed to the Windows clipboard. Choose one of three sizes or ***As shown***. ***Note:*** ***Small***, ***Medium*** *and* ***Large*** *copies use default image proportions.* ***As shown*** *retains the proportions as displayed on the screen.*
Save to file	Saves the panel as an illustration in selectable format.

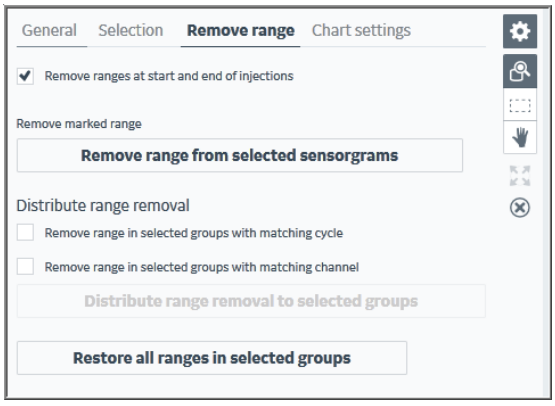
Selection tab



Note: *The curve markers shown here are examples, for illustration purposes only.*

Setting	Description
Exclude global	Check this option to exclude the selected data from all evaluation items, including those already created. Excluding data removes any sensorgram adjustments and/or table calculations that may have been applied.
Exclude local	Check this option to exclude the selected data from the current evaluation item only.
Show excluded data	Check this option to show excluded data in the sensorgram panel. Excluded sensorgrams are shown as gray lines.
Curve markers	Check the required markers to apply curve markers to the selected data.
Show curve markers	Check this option to show curve markers in the sensorgram and thumbnail panels.
Edit markers	Choose this option to manage curve markers.

Remove range tab (sensorgram sub-panel only)

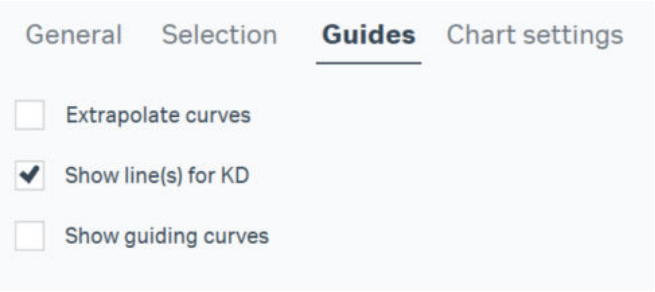


This action removes specified data ranges from selected sensorgrams. Drag the vertical markers to set the range that will be removed. Select one or more sensorgrams to activate the buttons. Ranges are removed in the current evaluation item only.

Note: *The vertical lines may not be visible if the sensorgram panel is zoomed.*

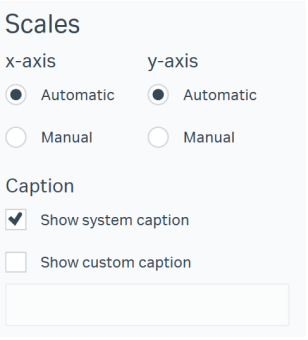
Setting	Description
Remove range at start & end of injections	When this option is checked, a fixed range (about 1 s) on either side of the sample injection start and stop events is removed, to avoid interference from disturbances that can occur at the injection event. ***Note:*** *This option affects all sensorgrams in all data series, regardless of selection.*
Remove range from selected sensorgrams	Select one or more sensorgrams, then click to remove the marked range from the selected sensorgrams. Only the selected sensorgrams in the current series will be affected.
Remove range in selected groups with matching cycle	Check this option to extend the selection to include sensorgrams with the same cycle number from all selected series. This option is primarily suitable for data with disturbances in multiple flow cells in the same cycle (1-channel data), or in parallel mode where different concentrations of the same sample are run in different channels in the same cycle (8-channel data).
Remove range in selected groups with matching flow cell/ channel	Check this option to extend the selection to include sensorgrams in the same flow cell (1-channel data) or channel (8-channel data) from all selected series. Use this option primarily in serial mode where different concentrations of the same sample are run in different cycles in the same flow cell/channel.
Distribute range removal to selected groups	Click to remove the marked range in accordance with the ***Distribute range removal*** setting.
Restore all ranges in selected groups	Click to restore removed ranges in the selected series. Removed data will be restored in all selected series, even if data was removed in several separate operations. ***Note:*** *You cannot restore ranges for individually selected sensorgrams.*

Guides tab (steady state sub-panel only)



Setting	Description
Extrapolate curves	If this option is checked, the fitted dose response curves are extrapolated to a wider concentration range.
Show line(s) for KD	If this option is checked, a KD line for each fitted curve is shown. The vertical line(s) is at the KD concentration. The line is red if the value is considered to be outside the range for confident measurement.
Show guiding curves	If this option is checked, dose response curves for fits using fitted R_{max} and constant R_{max}, as well as a horizontal line (dashed) corresponding to the expected R_{max}, are shown.

Chart settings tab



Setting	Description
Scales	Choose whether to use a logarithmic scale for the x-axis (applies to R_{eq} versus C plots only). Choose ***Automatic*** or ***Manual*** scales for the axes.

Setting	Description
Caption	Displays a caption in the panel. Choose whether to include the system caption and/or a custom caption with text as entered.

9.4.8 Fit details panel

Panel description

The ***Fit details*** panel holds tabs for ***Sample table***, ***Parameters***, ***Residuals***, ***Blanks***, and ***Quality control***. When the Biacore Intelligent Analysis extension is active, an additional ***References*** tab is added.

Quality control results are available only when the 1:1 binding model has been applied. The panel shows information for the last selected data series only.

The separate tabs are described below.

Sample table tab

The ***Sample table*** tab lists details of the cycles in the last selected data series.

Click ***Table settings*** on the panel toolbar to access settings for excluding selected cycles and assigning curve markers.

Parameters tab

The ***Parameters*** tab lists the fitting results and statistical parameters for the last selected data series. Parameters set to ***Global*** or ***Constant*** in the fitting model are listed as ***Series parameters***. Those set to ***Local*** are listed as ***Sensorgram parameters***. See *Appendix A Curve fitting procedures, on page 286* for more details.

Sample table | **Parameters** | Residuals | Blanks | Quality control | References

Steady state affinity Series parameters	
KD (M)	7.98e-09
Rmax (RU)	34.9
offset (RU)	2.3
Chi² (RU²)	2.19e+00
T(KD)	1.65e+00
T(Rmax)	6.09e+00
T(offset)	8.57e-01

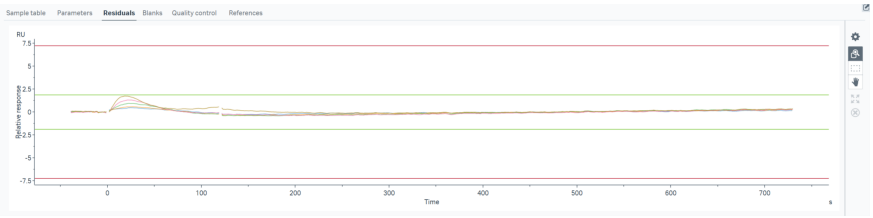
1:1 binding Series parameters	
ka (1/Ms)	2.41e+06
kd (1/s)	2.19e-03
Rmax (RU)	25.9
tc	9.24e+07
RI (RU)	0.0
Drift (RU/s)	0.00e+00
Chi² (RU²)	7.89e-02
T(ka)	8.83e+02
T(kd)	1.08e+03
T(Rmax)	3.26e+03
T(tc)	1.36e+02
T(RI)	Not a number
T(Drift)	Not a number
U-value	1

1:1 binding Sensorgram parameters		
Curve	Conc (M)	f (µl/min)
#5: Sensorgram Ch=1 Fc=2-1	1E-09	30
#6: Sensorgram Ch=1 Fc=2-1	2E-09	30
#7: Sensorgram Ch=1 Fc=2-1	4E-09	30
#8: Sensorgram Ch=1 Fc=2-1	8E-09	30
#9: Sensorgram Ch=1 Fc=2-1	1.6E-08	30

Residuals tab

The ***Residuals*** tab shows the residuals plot for kinetics fitting for the last selected data series. Residuals do not apply to steady state affinity fits. When the 1:1 binding model has been applied, colored lines are shown in the residual plot to indicate the range of acceptability. See *Appendix A Curve fitting procedures, on page 286* for more details.

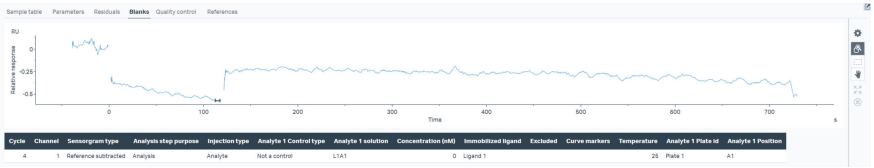
Click ⚙ ***Chart settings*** on the panel toolbar to access settings for the tab. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.



Blanks tab

The ***Blanks*** tab shows the blank cycles subtracted from the sensorgrams in the last selected data series.

Click ⚙ ***Chart settings*** on the panel toolbar to access settings for the tab. Other panel toolbar options are described in *General panel toolbar settings, on page 31*.



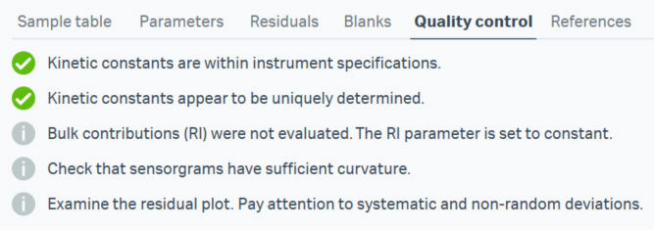
Follow the instructions below to change the way blank cycles are selected.

Action	Instruction
Change the general settings for blank cycles	Use the ***Blank settings*** in the ***Settings*** panel on the left of the workspace (see *Blank settings, on page 243*).
Exclude or include individual blank cycles	Select one or more sensorgrams or table rows in the ***Blanks*** tab, then use the ***Selection*** tab in ⚙ ***Chart settings*** or the right-click menu options.

Quality control tab

The ***Quality control*** tab gives a brief overview of the reliability of the results. Quality control results are available only when the 1:1 binding model has been applied.

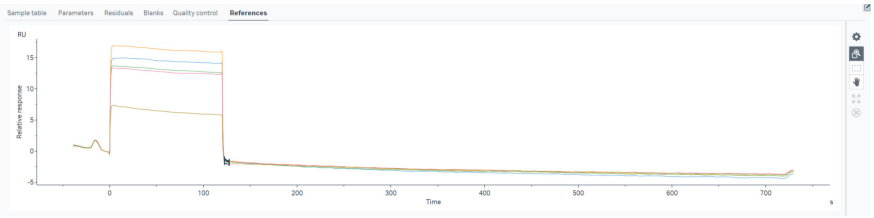
See *Quality control, on page 266* for more details.



Symbol	Description
(Green)	Pass: Quality assessment acceptable
(Orange)	Warning: Quality assessment close to the limits of acceptability
(Red)	Fail: Quality assessment unacceptable
(Gray)	User assessment recommended

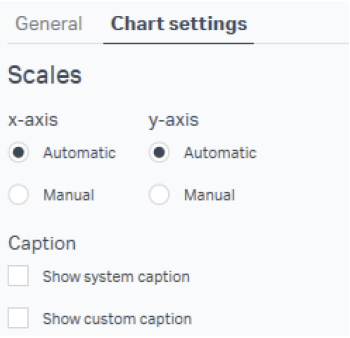
Reference tab

The reference tab, accessible with the ***Biacore Intelligent Analysis*** extension, shows binding to the reference surface for the selected series.



This panel has two settings:

Setting	Actions
General	• Copy. • Save to file.
Chart settings	Scales and caption choices, as shown in the below screen.



9.5 Kinetics and affinity item settings

Introduction

This section describes the available settings in the ***Settings*** panel at the left of the kinetics and affinity item workspace. The ***Select sensorgrams*** panel is described in *Workspace settings, on page 30*.

Data grouping

Data is grouped into data series for evaluation. All sensorgrams within a data series have the same interaction characteristics (analyte, ligand and injection conditions). In addition, the ***Data grouping*** setting determines how data from different cycles and channels is combined in data series for 8-channel systems according to the table below. The 1-channel systems are automatically set as ***Serial*** (see description of ***Serial*** in the table below) without the ***Data grouping*** option visible.

Setting	Description
Serial	Use when concentration series are run in different cycles within the same flow cell (1-channel data) or channel (8-channel data).
Parallel/2D	Use ***Parallel/2D*** grouping when concentration series are run in multiple channels, in either one (***Parallel***) or more (***2D***) cycles.

Note: *The grouping mode should be chosen in the first place by the way the run is set up (see the User Manual for the appropriate Biacore system). Runs that are set up in serial or parallel mode must be evaluated with the corresponding grouping. However, a run that is set up in 2D mode may be evaluated with either* ***Serial*** *or* ***Parallel/2D*** *grouping.*

Some grouping options may not be relevant, according to how the data is obtained.

Injection assignment

The ***Injection assignment*** setting determines which injections should be used for variable information and response values respectively.

For example, a run may take the sample name from the variable capture injection but the response for evaluation from an analyte injection.

Setting	Description
Use variable information from	Variable parameters from the specified injection command will be used as sample parameters in the evaluation.

Setting	Description
Use response values from	Response levels from the specified injection command in the run method will be used as sample responses in the evaluation.

Kinetics/Affinity mode

The mode setting determines which kind of evaluation will be performed for the selected series. The initial setting is determined by whether the item was created as ***Kinetics***, ***Affinity*** or ***Kinetics and Affinity***.

Changing the mode will delete any completed evaluations that are not within the scope of the new mode.

Note: *If you select multiple thumbnails where different thumbnails are assigned to different modes, the mode for the last selected thumbnail (with a heavy dark border) will be applied to all thumbnails in the selection.*

Mode	Description
Kinetics	Only kinetics evaluation will be applied. Changing to ***Kinetics*** mode will delete any previously performed affinity evaluation for selected series.
Affinity	Only steady state affinity evaluation will be applied. Changing to ***Affinity*** mode will delete any previously performed kinetics evaluation for selected series.
Both	Both kinetics and steady state affinity evaluation will be applied.

Blank settings

Blank cycles for kinetics and affinity evaluation are defined as sample cycles with zero concentration.

Choose how blank subtraction should be performed. The setting applies to the selected data series only.

Individual blank cycles can be excluded in the ***Blanks*** tab of the ***Fit details*** panel (see *Blanks tab, on page 239*).

Setting	Description
Use blanks within same series	Subtracts the median value of all blank cycles in the same data series.

Setting	Description
Use other blanks	Subtracts blanks according to the settings[1]: • ***Preceding*** subtracts the single nearest preceding blank • ***Following*** subtracts the single nearest following blank • ***Nearest*** subtracts the single nearest blank • ***Median consecutive preceding and following*** subtracts the median of the nearest set of consecutive blanks preceding and following the current cycle. Remove the checkmark from ***Keep within ligand*** to use blank cycles that use a different ligand.
No subtraction	Turns off blank subtraction.

[1] If ***Preceding*** or ***Following*** is selected, cycles that do not have a preceding or following blank respectively will not be subtracted.

Fit models

Different models and model settings are available for estimation of kinetics and affinity.

Choose the interaction model(s) for evaluating the data. The choice will apply to all currently selected data series. Models for kinetics, steady state affinity or both are available according to the ***Kinetics/Affinity mode*** for the current selection (see *Kinetics/Affinity mode, on page 243*). Changing the model after a fit has been performed will delete any previous fitting results of the same type (kinetics or affinity).

Click ***Perform fit*** to fit the chosen models to the experimental data. The number of data series for which fit will be performed is shown in parentheses.

The settings are described in the following table.

Setting	Description
Kinetics fit model ***Affinity fit model***	Select the fitting models to use for evaluation. See *Appendix A.2 Fitting models for kinetics and affinity, on page 289* for detailed descriptions.
Show affinity range (Affinity models only)	Check this option to show the data range used for determining the steady state response on sensorgrams. The steady state response is determined as the median response within the range.

Setting	Description
Calculate response at ... (Affinity models only)	Use these settings to adjust the data range used for determining the steady state response. Click ***Apply range*** to apply changes in the range settings.

Fitted curves and results are removed from data series in the following circumstances:

- When the ***Kinetics/affinity mode*** is changed. A change to ***Kinetics*** mode will remove any ***Affinity*** fits from the selected series and *vice versa*.
- When a new model is selected of the same type (kinetics or affinity) as the existing fit, including when parameters for constant R_{max} are changed.
- When initial values for the model used in the existing fit are changed.

Affinity range

The ***Affinity range*** settings are only visible with Biacore Intelligent Analysis, when there is an active quality prediction. They replace the ***Fit models*** settings. The settings apply to the selected data series only.

Choose from where the response is gathered for the K_D estimation and the size of the window. ***after injection start*** corresponds to the ***Affinity range position: Early*** setting in the ***Classification*** panel. Similarly, ***before injection end*** corresponds to ***Affinity range position: Late***.

The response value is calculated as the median response value in the window.

Initial values

Each fitting model requires settings and initial values for fitted parameters. The default settings are adequate for most purposes.

The settings and values may be changed in the table if desired. The table lists values for the currently selected model(s). Changes apply to all selected series that use the model for which the changes are made.

Initial values may be entered as numerical values or as expressions using functions evaluated within the data series (for example, ***Ymax*** is the maximum value on the y-axis, ***Ymax/5*** is the maximum y-value divided by 5).

Tip: *The default initial values for kinetic evaluation models set the bulk refractive index parameter to **Constant** with a value of zero. Change this setting to **Fit local** with a value of **Ymax/5** if your sensorgrams show significant bulk contribution.*

Note: *Changing the initial settings after a fit has been performed will delete the previous fitting results using the current model.*

Quality prediction

Quality prediction is only available when all of the following requirements are fulfilled:

- The ***Biacore Intelligent Analysis*** extension is active
- The ***Kinetics/Affinity*** mode is set to ***Affinity***
- The ***Steady state affinity*** model is selected in ***Fit models***
- Either ***after injection start*** or ***before injection end*** is selected for the position in ***Fit models***

Quality prediction enables automated analysis through machine learning prediction models. For more information, see *Section 9.6.1 Introduction, on page 248*.

Select ***Use prediction*** to activate the Quality prediction content.

The appropriate trained prediction model and model version can be selected from drop down lists.

Note: *It is not possible to use fragment affinity screen prediction models from Biacore Insight Evaluation Software version 4.0 or 5.0 in Biacore Insight Evaluation Software version 6.0.*

Evaluations where old models are used can still be opened.

Note: *The **Fit models** panel is replaced by the **Affinity range** panel when a prediction model has been applied.*

Click ***Train and save new version*** when changes have been made in the ***Classification*** or the ***Affinity range*** panels during the review process, to gradually change the prediction model to your preferences.

Training and saving new versions can only be performed on the latest available version of the prediction model.

Color by

The ***Color by*** setting colors sensorgrams and points according to a selected property. Available options are determined by variable parameters in the run method, as well as any sensorgram filters that are applied in the evaluation.

Note: *Sensorgram and point colors are hidden by the selection highlight when sensorgrams and points are selected.*

9.6 Biacore Intelligent Analysis for affinity screen evaluation

In this section

9.6.1 Introduction

Affinity screen is an assay to identify potential binders based on estimated affinity to the target. The ***Biacore Intelligent Analysis*** extension supports this assay, and also uses machine learning to provide input on whether to trust the results or not and suitable settings for the analysis of each compound. This can save time, provides confidence during evaluation, and reduces the risk of selecting leads with intrinsic problems such as non-specific binding and aggregation.

The ***Biacore Intelligent Analysis*** extension comes with predefined evaluation methods and a pretrained prediction model for the affinity screen. If you do not agree with the prediction, you can override it and train the model with your changes. This improves the prediction and adapts it better to your data, needs and interpretations in future evaluations.

For affinity screens, Biacore Intelligent Analysis does the following, in this order:

Stage	Description
1	Determines from which position of the sensorgram to extract the response.
2	Excludes deviating sensorgrams in a concentration series. Excluded sensorgrams deviate from the other sensorgrams in the series in one or several aspects, such as: • slope during injection • spikes in the area that is used for the dose response plot • deviating dissociation profile • deviating binding to the reference surface • response in seemingly wrong concentration order
3	Determines whether to use a steady state affinity model with fitted or constant R_{max}. The quality of the two fits and the position of the dose response curves in relation to the K_D values and the expected R_{max} value are considered.
4	Determines whether the compound and its K_D value is accepted or rejected. The series receives classifications describing why the results may not be trusted, such as atypical binding profile, binding to the reference surface, sub/super stoichiometric binding, low responses, or compound concentrations far from the estimated K_D concentration. Whether the series is accepted or rejected is based on the collective information of all its classifications. For severe classifications such as sub/super stoichiometric binding or concentrations far from K_D, a single classification is sufficient for a rejection.

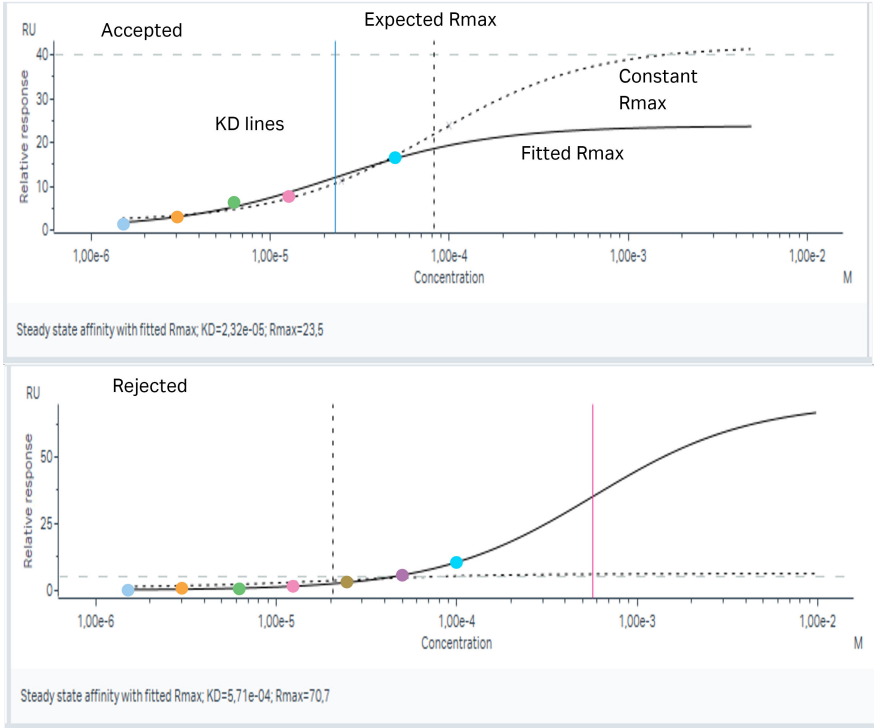
Biacore Intelligent Analysis for affinity screen evaluations is sometimes referred to as ***Quality prediction***.

Examples of accepted and rejected results with fitted R_{max}

In the graphs below, two dose response curves and two K_D lines based on fitted (solid line) or constant (dotted line) R_{max} are shown. The dashed horizontal line corresponds to the expected R_{max} value, i.e., the maximum response when all ligand binding sites are occupied. The vertical lines correspond to the estimated K_D values when a fitted (solid line) or constant (dashed line) R_{max} are used. The selected concentration (light blue) corresponds to the highest concentration in the series.

In the upper graph, concentrations are both above and below the K_D concentration when using the fitted R_{max} (solid vertical line). The fitted R_{max} dose response curve saturates below the expected R_{max} but it is not far enough for the data to be considered sub stoichiometric. Therefore, the series is accepted.

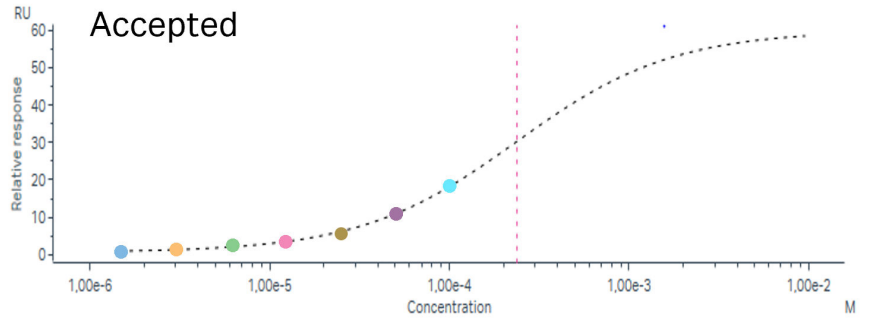
The lower data set should not be fitted with a constant R_{max} since the highest concentration is above the expected R_{max}. All concentrations are below the K_D concentration (solid vertical line). Based on the extrapolated dose response curve, the binding is considered super stoichiometric. This series is rejected.

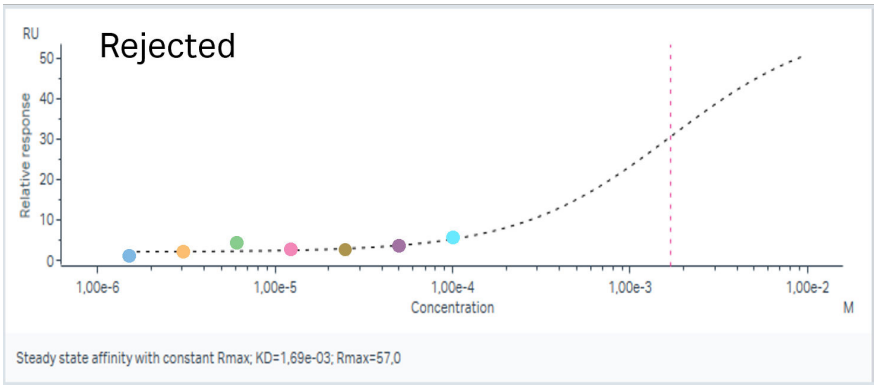


Examples of accepted and rejected results with constant R_{max}

Data fitted with constant R_{max} cannot be sub- or super stoichiometric since the dose response curves are forced to approach the expected R_{max} level.

In the graphs below, concentrations are all below K_D, but differs to their extent. The upper graph shows compound concentrations rather close to the K_D, causing an acceptance. The lower graph shows concentrations far below the K_D concentration, causing a rejection.





The steady state affinity model becomes rather rigid with a constant R_{max}. The prediction model is therefore trained to accept series with a larger difference between the included compound concentrations and the estimated K_D when a constant R_{max} is used.

9.6.2 Requirements on affinity screen runs

The following components are required for the predefined Biacore Intelligent Analysis evaluation methods for affinity screen:

- **R_{max} controls** are essential and used to calculate an expected R_{max} value for concentration series that are fitted using a constant R_{max} value. Ideally, the R_{max} control is run as a concentration series in all flow cells (1-channel data) or channels (8-channel data) with concentrations ranging from below K_D up to 15 times the K_D concentration. With this concentration series, a reliable R_{max} value can be determined. The molecular weight of the R_{max} control must be available and ***Analysis step purpose*** set as R_{max} control.

  One drawback with running a concentration series is that it consumes time and plate positions. Therefore, a single concentration can also be used as an R_{max} control. The concentration of that control must reflect the saturation level and must be 10 to 20 times higher than the K_D value.

  If no R_{max} control has been run, an R_{max} value can still be calculated provided that the ligand is immobilized, and that the immobilization level and the ligand molecular weight are available in the ***Home*** →***Properties*** item. The ligand is assumed to be 100% active and expected R_{max} values will be calculated for each sample using its molecular weight.

  Note: *The calculations will not be correct if the ligand is captured. Runs with captured ligands require an R_{max} control.*
- **Positive controls** are used to correct data for trends in ligand activity. The R_{max} control sample can be used as positive control but should be injected at a lower concentration (typically $5 \times K_D$) so that the response can reflect changes in ligand activity.
- **Negative controls** are used to check the consistency of data at zero analyte concentration.
- **Concentration series** for each sample, including at least one zero-concentration. The concentration information is also needed.
- **The MW** is used in combination with the response and MW of the R_{max} control to calculate an expected R_{max}.

Tip: *The predefined run method for fragment affinity screen includes the components listed above.*

9.6.3 Workflow for affinity screen evaluation using Biacore Intelligent Analysis evaluation method

This section describes the first way of evaluating affinity screen data with Biacore Intelligent Analysis: To use an evaluation method.

Follow the steps below to evaluate using an evaluation method for Biacore Intelligent Analysis.

Step	Action
1	Select one or several affinity screen runs in Biacore Insight Evaluation Software.
2	Apply a Biacore Intelligent Analysis evaluation method for affinity screen. Apply the solvent correction, if included in the method. *Result:* • Data is opened. • Sensorgrams with poor quality or deviating appearance are excluded. • K_D is estimated using settings that are predicted to be the most suitable. • The K_D of each series gets an acceptance state: ***Accept***, ***Reject,*** or ***Uncertain***. This is supported by classifications that describe the series.
3	Confirm that the positive controls and R_{max} controls are acceptable, found in the ***QC - Control binding*** and ***Evaluation - Rmax control*** items. Globally exclude any controls with deviating curve shape or amplitude from the right-click menu of the point or sensorgram. If any controls are excluded, perform another prediction from the ***Quality prediction*** setting (see *Quality prediction, on page 245* for more information).
4	(Optional) Clone the ***Evaluation - Affinity*** item before proceeding to the review process in step 5. This makes it possible to later compare the originally predicted result with the final results.
5	Review the predicted results. in particular any ***Uncertain*** results. If you do not agree with the predictions, change them in the ***Classification*** panel. See *Section 9.6.5 Review the results, on page 255* for more information. See *Section 9.8 Assessing kinetics and affinity results, on page 264* for general information about reviewing affinity results.
6	(Recommended) If a significant number of changes were made in step 5, train a new version of the prediction model from the ***Quality prediction*** setting. This adapts the model better to your data and definition of quality. See *Train and save new version, on page 260* for more information.

Step	Action
7	Find the compounds of interest, for example by selecting the ***Accepted*** tab in the result table and sort on ***KD*** from low to high values. See *Result table panel, on page 255* for more information.
8	Save the evaluation. ***Note:*** *In the predefined evaluation methods, the prediction model is set to version 1 in* ***Quality prediction****. To achieve a continuous training of models it is recommended to change the version to* ***Latest*** *when creating a new evaluation method.*

9.6.4 Workflow for affinity screen evaluation using manual application of Biacore Intelligent Analysis

This section describes the second way of evaluating affinity screen data with Biacore Intelligent Analysis: To apply the prediction manually.

Follow the steps below to manually apply a prediction to already opened data.

Step	Action
1	Confirm that the run contains all necessary controls and that required information is available in the ***Variables*** workspace. See *Section 9.6.2 Requirements on affinity screen runs, on page 251* for more information.
2	Globally exclude any positive controls with deviating curve shape or amplitude from the right-click menu of the point or sensorgram. Depending on evaluation method, controls may be found in the ***QC - Control binding item***. If not, create a new ***Plot*** item and display the controls.
3	Create a new ***Affinity*** item in the ***Home*** workspace.
4	Select a prediction model and perform the prediction from ***Quality prediction*** in ***Settings***. See *Quality prediction, on page 245* for more information.
5	Perform steps 4-8 described in *Section 9.6.3 Workflow for affinity screen evaluation using Biacore Intelligent Analysis evaluation method, on page 252*.

9.6.5 Review the results

It is recommended to review the results of the prediction, to ensure a high quality of the evaluation. In particular:

- Go through the ***Uncertain*** results and define whether they should be accepted or rejected. These are found in a separate tab in the result table. When you have a model that you trust, these results may be the only ones needing attention.
- Confirm that the sensorgram exclusion is reasonable, or make any necessary changes.
- Edit the fit settings if you do not agree with the predicted settings.
- Edit the predicted acceptance state or classifications if you do not agree with any of the predictions.

Changes to predictions are done in the ***Classification*** panel and apply to selected data. See the following information about each panel for details about the review process, and *Examples of accepted and rejected results with fitted Rmax, on page 249* for tips.

Note: *All manual changes are discarded if you perform another prediction.*

Note: *Excluding sensorgrams or changing the fit settings causes an automatic refit of the data. The acceptance state is not affected by sensorgram exclusion.*

Result table panel

At the top of the ***Result table***, there are four tabs; ***Accepted (mn)***, ***Rejected (mn)***, ***Uncertain (mn)***, and ***All (mn)***, where mn is the number of samples in each category.

The currently selected tab is displayed with bold text. The tabs function as filters and by selecting a tab, the thumbnails and result table are updated to show only the corresponding series.

The table below describes columns specific for ***Quality prediction*** results when the ***Biacore Intelligent Analysis*** extension is active. If they are not visible, add them as described in *Columns tab, on page 223*.

Column	Description
Affinity range position	The position of the response range used for calculating the affinity, either early or late during the analyte injection.
Rmax type	The type of R_{max} setting used for calculating the affinity, either constant or fitted.

Column	Description
Classification	For each series, the classifications are listed. Series can have 0, 1, or several classifications. The classification column can be filtered to show series with selected classifications. The filter funnel turns blue when a filtration is active. See *Classification panel, on page 257* for details.
Acceptance certainty (%)	The ***Acceptance certainty*** is calculated by the prediction model and is scaled between 0% to 100%. State ***Accept*** scores close to 100% when the set is without detrimental classifications. An ***Uncertain*** state is approximately 50%, and ***Reject*** state is close to 0%.
Rmax/Expected Rmax	The R_{max} value from the fit is compared to the expected R_{max} value. The expected R_{max} value is based on an R_{max} control, or on the assumption that the immobilized ligand is 100% active. If an R_{max} control is not available, the immobilization level and molecular weight of the ligand must be available.
KD (fitted Rmax)/KD (constant Rmax)	The ratio between the K_D values estimated with the fitted R_{max} and constant R_{max} affinity fit models.
Baseline difference	The baseline difference between the following cycle and present cycle. A high value can indicate that the sample blocks the target, which can impact binding in subsequent cycles.
Offset/Expected Rmax (%)	A calculated column which shows the offset of the fitted curve in comparison to expected R_{max}.

Sensorgrams panel

The ***Sensorgrams*** panel is divided into two sub-panels, showing the sensorgrams on the left and the steady state response plotted against concentration on the right. The panel displays the most recently selected series.

Concentration-response curves for both a fitted (solid line) and a constant R_{max} (dotted line) are visible in the plot on the right, with corresponding vertical lines representing their K_D values. Which K_D that is reported for the series is defined by the ***Rmax type*** setting and this line is blue, or red if the concentration range does not cover K_D. The estimated R_{max} is shown as a dashed horizontal line.

Classification panel

The ***Classification*** panel is accessible with the ***Biacore Intelligent Analysis*** extension, when ***Quality prediction*** is active (set to ***Use prediction***).

Show/Hide the ***Classification*** panel by clicking on the icon. It is divided into three sections:

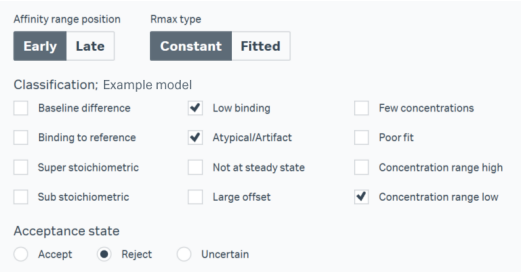
- Settings for the affinity fit.
- ***Classification*** of sensorgrams and concentration series.
- ***Acceptance state*** settings. All series that are rejected always have at least one classification that motivates its rejection.

See *Examples of accepted and rejected results with fitted Rmax, on page 249* for tips and examples for reviewing affinity screen data.

The ***Classification*** panel presents predicted fit settings, classifications and acceptance state, which can be changed from the panel. Changing the ***Acceptance state*** automatically transfers the series to the tab of its new state in the ***Result table***.

Note: *Classifications may have to be changed if the **Rmax type** setting is changed.*

The ***Classification*** panel can be used with a single concentration series. It is also possible to select and classify several series at the same time. The ***Classification*** panel shows the settings of the last selected concentration series.



The table below describes the possible settings of the ***Classification*** panel, using the default names of the classifications. The names presented in the ***Classification*** panel are the ones defined when creating the selected prediction model (see *Section 3.6.1 Panel description, on page 52*).

Note: *Available classifications and the classification names are slightly different if a prediction model from Biacore Insight version 4.0 or 5.0 is used.*

Setting/Classification	Description
Affinity range position	The position of the response range used for calculating the affinity, either early or late during the analyte injection. The position is predicted by the model but can be changed manually, to better reflect the data or to avoid injection disturbances. A more detailed adjustment can be done from the ***Affinity range*** settings. ***Note:*** *Changing the affinity range position causes the data to be refitted. The acceptance state remains the same as before the change.*
Rmax type	The type of R_{max} setting used for calculating the affinity, either constant or fitted. It corresponds to the ***Fit*** setting in ***Initial values***. The R_{max} type is predicted by the model but can be changed manually. ***Note:*** *Changing the R_{max} type causes the data to be refitted. The acceptance state remains the same as before the change.*
Baseline difference	Large difference in the baseline levels between the current cycle and the next cycle.
Binding to reference	The remaining binding to the reference surface after the analyte injection is significant.
Super stoichiometric	The series has a fitted R_{max} that is much higher than the expected R_{max}.
Sub stoichiometric	The series has a fitted R_{max} that is much lower than the expected R_{max}.
Low binding	The binding responses are very low.
Atypical/Artifact	The majority of the sensorgrams show one or several of the following: significant drift, large offsets, signals below baseline during the dissociation phase, the dissociation is slow and shows no sign of reaching the baseline, negative response.
Not at steady state	One or several sensorgrams in the series do not approach steady state response during the injection.
Large offset	The offset of the fitted curve is large in comparison to R_{max}.
Few concentrations	The number of data points in the fit are few.

Setting/Classification	Description
Poor fit	The fitted curve fits poorly to the data points.
Concentration range high	The analyte concentration range is high in relation to the affinity.
Concentration range low	The analyte concentration range is low in relation to the affinity.
Concentrations above or below KD	All of the concentrations are above/below K_D. ***Note:*** *This classification only appears when the prediction model is from Biacore Insight software version 4.0 or 5.0.*
Constant Rmax	The type of R_{max} used for calculating the affinity is set to constant. ***Note:*** *This classification only appears when the prediction model is from Biacore Insight software version 4.0 or 5.0.*
Acceptance state: Accept	The K_D value is accepted.
Acceptance state: Reject	The K_D value is rejected.
Acceptance state: Uncertain	It is not possible to assign ***Accept*** or ***Reject***.

Fit details panel

A curve can be excluded or included from the right-click menu either from the row in the ***Sample table*** or from the right click menu in the ***Sensorgram*** panel (when a sensorgram is selected).

Note: *Excluding sensorgrams from a series, also any associated blanks, causes an automatic refit of the data. The acceptance state is not affected by sensorgram exclusion.*

The ***Reference*** tab shows binding to the reference surface for the selected series.

9.6.6 Managing prediction models

The pretrained model for affinity screen can be used, and potentially further trained, if it seems to predict your data well. It is also possible to create your own prediction model, which is done in the ***Prediction model*** workspace (see *Section 3.6 Prediction models, on page 51*).

Base your new model on the pretrained model if it seems to predict your data relatively well. If the generated data differ much from the data that the model was trained for, or if different adjustments are done than the recommended ones, it may be less suitable. In those cases, it is better to base the new model on an empty model. This is because an empty model quickly adapts to training, while the pretrained model needs more input to significantly change. An empty prediction model must be trained before it can be used to predict results.

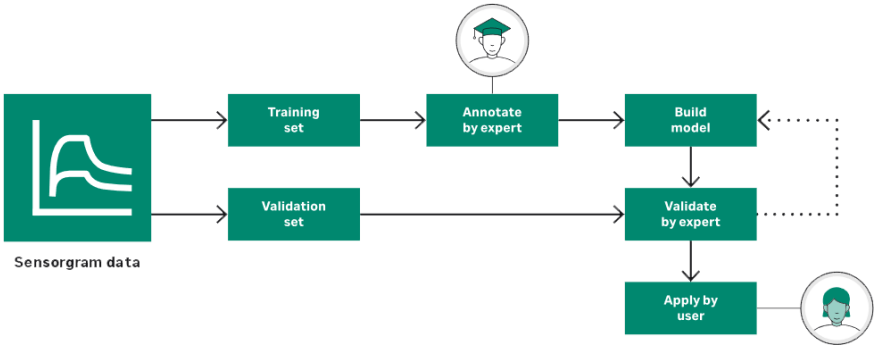
Note: *The original pretrained models can only act as template for other models, but not be used in prediction. This is to ensure that they are not accidentally overwritten upon training. In the predefined evaluation methods, copies of the original models are used instead.*

Train and save new version

The ***Train and save new version*** button located in the ***Quality Prediction*** setting becomes activated when a change in the ***Classification*** panel has been made. The prediction model is trained by clicking ***Train and save new version***, and the version number of the prediction model is incremented by one. Training a prediction model gradually customizes it to your preferences, and is always performed using the latest version of the prediction model.

Principle of Biacore Intelligent Analysis

The image and text below describe how the pretrained Biacore prediction model for binding level screen was originally created. The same approach may be used when training other models, to confirm that they perform as expected.



To train the model, data were divided into a training and a validation set. The training set was annotated by an expert who assigned classifications and quality levels to each sample. This input was used for training the model, which was then applied to the validation set.

The predictions of the validation set were examined by an expert and if the results were not satisfactory, the model continued to be trained with new data until the predictions of the validation set were at a satisfactory level.

9.7 Selecting data in kinetics and affinity items

Introduction

This section describes how to select and manage data in ***Kinetics*** and ***Affinity*** items.

Coordinated selections

Selection is coordinated between different panels so that selecting or deselecting in one panel automatically selects or deselects in coordinated panels. Panels are coordinated separately for data series and data within a series. For example, selecting a single sensorgram in the ***Sensorgram*** panel does not affect the selection in the ***Thumbnails*** panel. The table below shows how panels are coordinated.

Panel content	Coordinated panels
Data series	***Thumbnails*** ***Result table*** ***Classification*** ***KD chart*** ***On-off rate chart***
Data in one series	***Sensorgram*** (both subpanels) ***Fit details***: ***Sample table*** tab ***Fit details***: ***Sensorgram parameters*** on ***Parameters*** tab ***Fit details***: ***Residuals*** tab ***Fit details***: ***References***

Selection in the *Thumbnails* panel

Click the ***Select all thumbnails*** icon to select all thumbnails. Data series selection in the ***Thumbnails*** panel behaves differently according to whether ***Selection as toggle*** is checked in the thumbnail settings. (See also *Panel toolbar, on page 217*.)

Selection as toggle	Description
Unchecked	Click a thumbnail to select it. Any previously selected thumbnails will be deselected. Use **Shift-click** and **Ctrl-click** to select multiple thumbnails.
Checked	Click a thumbnail to toggle between selected and deselected. The status of other thumbnails is not affected.

All selected thumbnails are marked with a heavy light or dark blue border. When multiple thumbnails are selected, the most recently selected thumbnail is marked with a heavy dark border, and details of that series are shown in the ***Sensorgram*** and ***Fit details*** panels. ***Kinetic/Affinity*** mode, ***Initial values***, and fitting settings and actions are applied to all selected series.

Selection in the ***Thumbnails*** panel is coordinated with selection in the ***Result table***, ***On-off rate chart***, and the ***KD chart***.

Tip: *To browse through the selected thumbnails without changing the selection, choose* ***Selection as toggle*** *and then click twice on a thumbnail with a light blue border. The first click will deselect the thumbnail, the second will select it with a heavy dark border. Using* **Ctrl-click** *has the same effect regardless of the* ***Selection as toggle*** *status.*

Selection in the *Result table*, *On-off rate chart* and *KD chart* panels

Selection of rows in the ***Result table*** panel or points in the ***On-off rate chart*** and ***KD chart*** panels is coordinated with selection in the ***Thumbnails*** panel, and which result the ***Classification*** panel applies to.

Note: *The* ***Selection as toggle*** *setting applies only to thumbnail selection and does not affect selection in other panels.*

Selection in the *Sensorgrams* panel

Click on a sensorgram or point to select or deselect it.

Use ***Select area mode*** (see *General panel toolbar settings, on page 31*) to select multiple sensorgrams or points in one operation.

Click to deselect all selected sensorgrams.

Note: *Only experimental curves can be selected.*

Selection in the ***Sensorgram*** panel is coordinated with selection in the ***Fit details*** panel.

Note: *For single cycle affinity runs, selecting one point in the plot of response against concentration will select all points. Single points cannot be selected in this type of run.*

Selection in the *Fit details* panel

Selection in the ***Sample table***, ***Parameters***, and ***Residuals*** tabs in the ***Fit details*** panel is coordinated with selection in the ***Sensorgram*** panel.

9.8 Assessing kinetics and affinity results

Introduction

This section describes how to assess the quality and reliability of kinetics and affinity evaluations.

Magnitude of reported parameters

Check that the magnitude of the reported parameters is reasonable. Reported parameters represent mathematical solutions to the curve fitting, without regard to what is reasonable for a molecular interaction or what can be measured in the Biacore system from which the results were obtained. In particular, parameters that are not significant for the fitting (see *Standard error and T-value, on page 265*) may have any value, often unreasonable.

Kinetic and affinity constants that are close to or outside the range that can be measured in the instrument should be treated with caution. Typical working ranges are given in the data sheet for the respective instruments.

Chi-square

Chi-square is a measure of the average squared residual (the difference between the experimental data and the fitted curve), and is an indicator of how closely the fitted curves agree with the experimental data. A lower chi-square indicates closer fitting. One chi-square value is reported for the whole fitting.

Chi-square is calculated as

$$\text{chi-square} = \frac{\sum_{1}^{n}(r_f - r_x)^2}{n - p}$$

Parameter	Description
r_f	Fitted value at a given point
r_x	Experimental value at the same point
n	Number of data points
p	number of fitted parameters

For sensorgram data used in kinetic fitting, the number of data points is very much larger than the number of fitted parameters in the model, so ***(n-p)*** approximates to ***n***, and the chi-square value approximates to the average squared residual per data point. This does not hold for affinity data, where the number of data points is the same order of magnitude as the number of fitted parameters.

Chi-square should be assessed in relation to the response levels in the sensorgrams. Higher response values will generate higher chi-square values for the same relative deviation from the fitted curve.

Chi-square is listed in the ***Result table*** panel and on the ***Parameters*** tab in the ***Table*** panel.

Standard error and T-value

The standard error (SE-value) is an indicator of the significance of a fitted parameter, and represents an estimate of how much the parameter value can vary without significantly affecting the closeness of fit. As a general rule, if the SE-value for a fitted parameter is 10% or less of the reported parameter value, the value can be regarded as significant. Parameters that have an SE-value of the same order of magnitude as or higher than the parameter value itself can vary widely without significantly affecting the closeness of fit, and should be treated with caution. Typically (but not always), parameters with a low significance may have unreasonable values.

Note: *Even if parameters with low significance can have a wide range of values without affecting the fit, repeated evaluation of the same data set will always return the same value. Consistency of a value between repeated evaluations is* ***not*** *a test of significance.*

The T-value is obtained by dividing the parameter value by the standard error, and represents an expression of the standard error normalized with respect to the parameter value. High significance is thus indicated by a low standard error or a high T-value. Approximate guidelines for interpreting T-values are given in the table below.

T value	Significance
<1	The parameter is not significant for the fitting and the value cannot be reliably determined from the experimental data.
1 to 10	The parameter significance is low but should not be ignored.
>10	The parameter is significant for the fitting and the value may be regarded as reliable.

The SE-value and T value for each fitted parameter can be included as columns in the ***Result table*** panel for a completed fit (see *Columns tab, on page 223*). Columns are specific to each fitting model. The T-value is also shown for each fitted parameter on the ***Parameters*** tab in the ***Fit details*** panel.

U-value

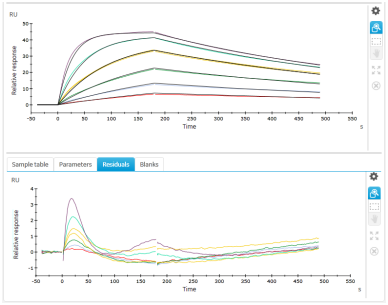
The U-value is an estimate of the uniqueness of the calculated values for rate constants and R_{max}. If parameters are correlated, the fitting procedure can determine their relative magnitudes but not absolute values (for example, knowing the affinity gives the ratio but not the values for rate constants). The U-value is determined by testing the dependence of the fit on correlated variations in pairs of parameters, and is reported on the ***Parameters*** tab in the ***Fit details*** panel, as a single value for the whole fitting. U-values above about 25 indicate that absolute values for two or more of the parameters (rate constants and R_{max}) are correlated and cannot be determined. If the U-value is below about 15 the parameter values are not significantly correlated.

The U-value is listed in the ***Result table*** panel, and on the ***Parameters*** tab in the ***Fit details*** panel.

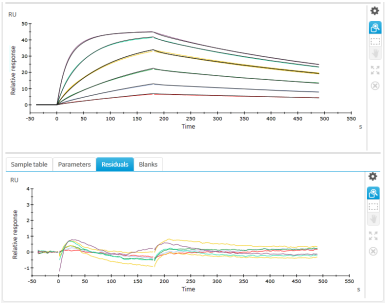
Residuals

Residuals show the difference between experimental and fitted value for each data point in the sensorgrams. For a perfect fit, the residuals scatter randomly around zero. Systematic deviations, seen as a definite shape in the residual plot, indicate that the interaction model is to a greater or lesser extent unsuitable for the interaction.

The illustrations below show the same data fitted to two different models, giving residuals that are less acceptable (left) and more acceptable (right).



Residuals for a poor fit show strong systematic deviations from zero.



Residuals for a good fit are close to zero even if small systematic deviations may be present.

Quality control

When the 1:1 binding model has been used for kinetic fitting, the quality of the fit is assessed in terms of the following factors, described in the following sections. Quality control results are listed on the ***Quality control*** tab in the ***Fit details*** panel.

- Magnitude of kinetic constants
- Parameter uniqueness
- Bulk refractive index contributions
- Sensorgram curvature

- Residuals

Magnitude of kinetic constants

If either association or dissociation rate constants are close to or outside the limits that can be determined in the instrument, this will be reported. For values close to the limit, judge the validity of the results on other assessment criteria as described in this chapter.

Parameter uniqueness

In some situations, it may be possible to determine a value for two or more parameters in combination without being able to determine unique values for the individual parameters. Such parameters are said to be correlated. One example is the pair of kinetic rate constants k_a and k_d, that are related through the affinity constant K_D ($K_D = k_d/k_a$): it may be possible to determine the affinity constant reliably without being able to resolve the individual rate constants. Parameter uniqueness is assessed by testing correlation between pairs of the parameters k_a, k_d and R_{max}. If significant correlation is found, this will be reported as a warning that parameters cannot be uniquely determined.

Note: *This test does not explore all possible parameter correlations. A Pass status for this test is not a fail-safe indication that parameters are uniquely determined.*

Bulk refractive index contributions

After reference subtraction and blank subtraction, sensorgrams for kinetic evaluation should not in principle contain any bulk refractive index shifts (parameter RI in predefined models). However, there may be some circumstances where small bulk refractive index shifts may remain in reference and blank-subtracted data. On the other hand, the fitting algorithm tends to interpret rapid interaction events (incorrectly) as bulk shifts. If the fitting returns significant values for RI, a warning will be issued in the quality control tab. Examine the sensorgrams and fitted curves to determine whether bulk shifts as reported by the fitting are true or false. In cases where reported bulk shifts are unreasonably large, you may want to set RI to a constant value of zero in the Parameters setting for the fitting. If you do this, the bulk contributions component in the quality control tab will be reported as neutral since the RI parameter was not evaluated.

Sensorgram curvature

You should check that the sensorgrams have sufficient curvature for kinetic determination. Ideally, the sensorgrams for at least the one or two highest concentrations should show measurable binding rates at the beginning of the sample injection and approach a steady state towards the end of the injection. Sensorgrams that approximate to "square-wave" pulses (indicating rapid association and dissociation) and those that do not flatten out during the injection generally do not contain sufficient kinetic information for reliable evaluation. Ideally, the dissociation phase should be long enough to monitor a fall in response of at least 10-15% of the starting value.

If the interaction is too fast to provide kinetic information, you may only be able to determine affinity constants. Interactions that do not flatten out sufficiently during the injection or dissociate sufficiently during the dissociation phase may sometimes be analyzed by prolonging the association or dissociation phase respectively.

Residuals

You should check that the residuals (the difference between experimental and fitted value for each data point in the sensorgrams) lie within reasonable limits. For a perfect fit, the residuals reflect the short-term noise in the sensorgrams and scatter around zero (typically ±1-2 RU). Systematic deviations, seen as a definite shape in the residual plot, indicate that the interaction model is to a greater or lesser extent unsuitable for the interaction. As an aid in judging the residuals, guidelines are drawn on the residual plot to indicate the range of acceptability. Most of the residuals should be within the inner (green) limits. The guideline positions are calculated in relation to the response range of the sensorgrams.

Considerations for steady state affinity

Steady state affinity evaluations are performed by fitting a plot of R_{eq} against concentration C to a model representing equilibrium 1:1 binding. The closeness of fit is reported as a chi-square value, calculated in the same way as for kinetics. However, the number of points in the steady state affinity plot is very much lower than for kinetic evaluation, which makes chi-square a more sensitive indicator of fitting quality.

The plot of R_{eq} against C approaches a limiting value (equivalent to R_{max}) at high concentrations. Robust evaluation of the data requires either that the plot shows sufficient curvature for reliable estimation of R_{max} or that a valid constant value is provided for R_{max}. As a rule of thumb, evaluation with fitted R_{max} is acceptable only if the calculated K_D value is less than half the highest analyte concentration used. (For a 1:1 interaction, the K_D value is equal to the analyte concentration that gives 50% saturation of the binding sites, so that $R_{eq} = 0.5 \cdot R_{max}$. In other words, reliable evaluation is only obtained if the surface is more than 50% saturated at the highest analyte concentration.)

For weak interactions where it is not possible to use concentrations approaching $0.5 \cdot R_{max}$, evaluation using a model with constant R_{max} may give more reliable results. This approach requires that R_{max} is determined separately, for example using high concentrations of a known binder.

10 Exporting data

In this chapter

Introduction

Evaluation results can be manually exported in the following formats:

- As a Microsoft Excel spreadsheet, suitable for further processing in third party applications.
- As a Microsoft PowerPoint file, suitable for presentation purposes.
- As a PDF document, suitable for archiving purposes.
- As a JSON file or an XML file, suitable for interchange of data between different software. These two formats are available when the ***Data Integration*** extension is active.

Manual export in all formats is performed from the ***Home*** workspace.

Note: *Items that are flagged for possible changes will be updated automatically when they are exported (see Updating items, on page 28). It is recommended to update and check all items before performing an export operation.*

Runs and evaluations can also be automatically exported in JSON format to a third-party software via the Biacore Insight API (Application Programming Interface).

The purposes of such automatic exports can be:

- Centralized data management, in a LIMS system or similar.
- Data evaluation in company-specific software.
- Data archiving.

Export via the API requires an active ***Data integration extension***. A separate API server must be installed and configured and an application that requests and handles data must be developed. More detailed information for IT personnel installing the API server and integrating with the Biacore Insight API is found in the *Biacore Insight API Installation and Management Guide 29798347*.

In addition, database objects (runs, evaluations, methods, and evaluation methods) can be exported in a proprietary format for transfer to another Biacore Insight database.

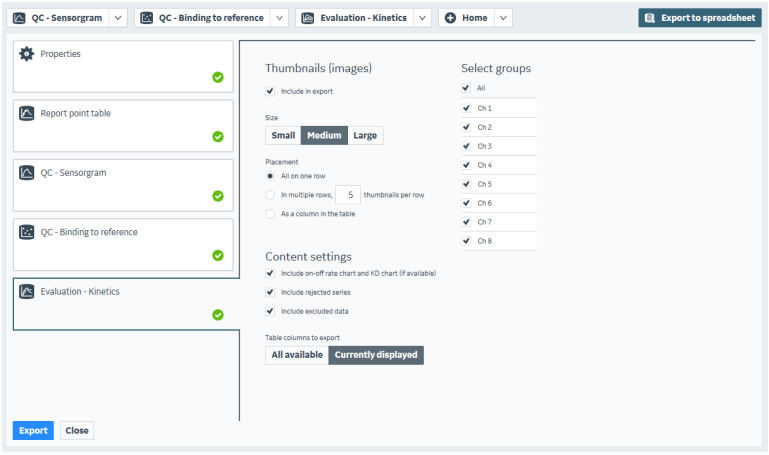
10.1 Export to spreadsheet

Introduction

This function exports all or selected items from the current evaluation session to an external file in Microsoft Excel format (`.xlsx`). Each component in the results is exported to a separate worksheet in the Excel workbook. Full details of each item are exported, regardless of how much is displayed on the screen.

Procedure

Follow the steps below to export evaluation results in spreadsheet format.

Step	Action
1	Go to the ***Home*** workspace and select ***Spreadsheet***. *Result:* The ***Export to spreadsheet*** workspace opens. 
2	Remove the checkmark from components that you do not wish to export.
3	Work through the components selected for export and adjust the settings to suit your needs. Settings differ for different kinds of component.
4	Select ***Export*** and provide a file name. You can choose to view the exported file directly in Excel.

Exported details

The exported components can include a ***Report Point Table***, with full details of all report points in the evaluation. This table includes information that is not accessible from within the software. The following statistical parameters are included:

Column	Description
Standard deviation	Standard deviation of data points in the report point window, calculated as $SD = \sqrt{\frac{1}{(n-1)}\sum(y-\bar{y})^2}$ where n = number of points and y = response in RU
Slope (RU/s)	Slope during report point window in RU s^{-1}, calculated as $slope = \frac{\sum(y-\bar{y})(x-\bar{x})}{\sum(x-\bar{x})^2}$
LRSD	Alignment of slope to a straight line (regression coefficient), calculated as $LRSD = \sqrt{\frac{Q_0}{(n-2)}}$ where $Q_0 = \sum(y-\bar{y})^2 - \frac{(\sum(y-\bar{y})(x-\bar{x}))^2}{\sum(x-\bar{x})^2}$

10.2 Export to presentation or PDF

Introduction

These functions export all or selected data from the current evaluation session to an external file in Microsoft Powerpoint (`.pptx`) or PDF format.

Scope

Evaluation items (***Sensorgram***, ***Plot***, ***Concentration***, ***Epitope binning***, ***Kinetics and affinity***, ***Kinetics***, and ***Affinity***) can be exported according to the table below. Preparation items such as ***Variables***, ***Curve markers***, ***Report points*** and ***Solvent correction*** are not exported.

Item	Panel	Comments
Sensorgram	***Thumbnails***	Exported as shown in the item.
	Sensorgrams	Exported as shown in the item.
	Table	Exported as shown in the item.
Plot	***Thumbnails***	Exported as shown in the item.
	Plot	Exported as shown in the item. Sensorgrams for selected plot points are not exported.
	Table	Exported as shown in the item.
Concentration	***Thumbnails***	Exported in vertical groups of three as displayed.
	Plots	Exported as shown in the item. Each plot panel is exported to a separate page. Sensorgrams for selected plot points are not exported.
	Parameters	Exported as shown in the item.
	Table	Exported as shown in the item.
Epitope binning	***Thumbnails***	Exported as shown in the item.
	Sensorgrams	Exported as shown in the item. Each sensorgram is exported to a separate page.
	Heat maps	Exported as shown in the item. Each heat map is exported to a separate page.

Item	Panel	Comments
	Bin charts	Exported as shown in the item. Each bin chart is exported to a separate page.
	Table	Exported as shown in the item.
Kinetics and affinity ***Kinetics*** ***Affinity***	***Thumbnails***	Exported as shown in the item.
	Result table	Exported as shown in the item.
	KD chart	Exported as shown in the item.
	On-off rate chart	Diagonal (constant K_D) lines are not shown.
	Sensorgrams	Exported as shown in the item. Exported as kinetic sensorgrams if the thumbnails are in ***Sensorgrams*** mode. Exported as plots of ***Req*** against ***C*** plots if the thumbnails are in ***Plot*** mode.

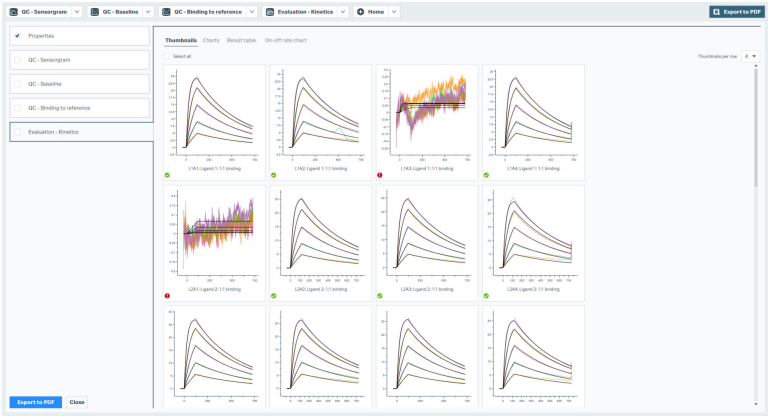
Procedure

Follow the steps below to export the results. Settings for exporting to ***Presentation*** and ***PDF*** are independent of each other.

Note: *Some steps in the export procedure may take several minutes depending on the amount of data and the available computer memory.*

Step	Action
1	Choose the display setting for the thumbnails in the items to be exported. Exported thumbnails will use the same settings as in the item.
2	Choose and arrange the columns in the ***Table*** panel of the items to be exported. By default, all columns displayed in the item panel are exported to the presentation. Columns can be removed from the exported data, but cannot be added or rearranged once the export operation has been selected. All table rows are included in the exported data by default. Row selection in the item panel does not affect rows in the exported data.
3	Go to the ***Home*** workspace and select ***PDF*** or ***Presentation***.

Step	Action
	Result: The ***Export*** workspace opens. Components of each evaluation item are shown on separate tabs.

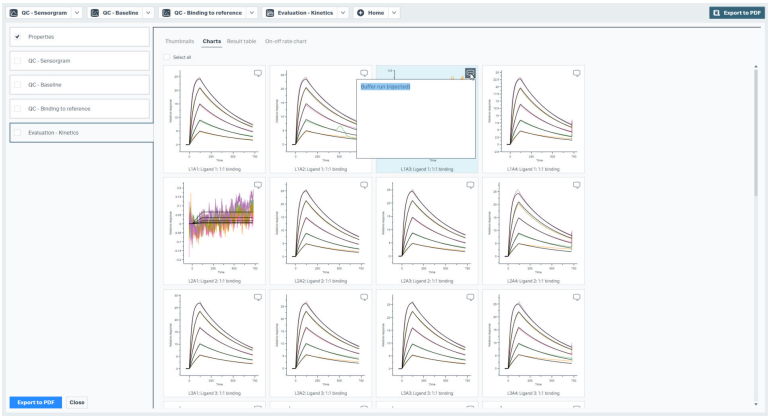


4 In ***Properties***, select whether to include the ***Evaluation properties*** and/or ***Run properties*** in the exported file. The method summary is included in run properties.

Note:
*The **Notes** are exported as a separate slide in a presentation or a separate page in a PDF file.*

5 Choose the evaluation items to be exported. For each item, choose the details of the thumbnails, charts and table.

6 For ***Thumbnails***, choose the number of columns for thumbnail display in the exported file.

Step	Action
7	For ***Charts***, click 🗨 to add an optional comment. 
8	For ***Tables***, exclude table rows by removing the checkmark from the row in the table preview. To exclude columns, remove the checkmarks from the ***Columns***.
9	Select the ***Export...*** button at the bottom left and provide a location and name for the exported file. *Result:* The evaluation is exported.

10.3 Export to JSON or XML

Introduction

The JSON and XML export formats are only available when the ***Data Integration*** extension is active.

Procedure

Follow the steps below to export evaluation results in JSON or XML format.

Step	Action
1	Go to the ***Home*** workspace and select ***JSON or XML***. *Result:* The separate window ***Export evaluation to JSON or XML*** opens.
2	Navigate to the destination folder and verify or adjust the suggested file name.
3	Select file format and click ***Save***. ***Note:*** *For JSON, it is possible to choose between the previous file format (that was used in Biacore Insight Software version 5.0) and the latest file format, that is also used for API exports.*

For automatic export via the API, refer to the *Biacore Insight API Installation and Management guide 29798347*.

10.4 Transfer to another Biacore Insight database

Introduction

Objects from the Biacore Insight database (runs, evaluations, methods, and evaluation methods) can be exported in a proprietary format for transfer to other Biacore Insight databases.

Procedure

Follow the steps below to transfer a database object to another Biacore Insight database.

Operation	Instruction
Export a single object	1. Scroll to the right if necessary. 2. Select ***Export*** in the ***Action*** column. The file is exported in a proprietary format intended for import to another Biacore Insight database.
Export multiple objects	1. Select a folder or perform a search to define the objects to be exported. All objects shown on the current page will be included in the export. 2. Choose ***Show more*** in the right-hand panel of the workspace. 3. Click ***Export objects***, and select whether you want all objects in the same folder, or if you want the folder structure to be preserved. The files are exported in a proprietary format intended for import to another Biacore Insight database. ***Tip:*** *Use the search function to export objects of different types (runs, run methods, evaluations, evaluation methods) in a single operation.*

Operation	Instruction
Import object(s)	1. Navigate to the destination folder in the database. 2. Select  ***Import*** from the icons at the bottom of the panel. 3. Select file(s) to import. Supported file types are files exported from a Biacore Insight database and result files (.blr) from certain other Biacore systems (currently Biacore S200 and Biacore T200). ***Note:*** *Imported .blr files can only be opened in the Biacore Insight Evaluation Software.*

11 Evaluation methods

Introduction

Evaluation methods provide a way to standardize the steps performed in an evaluation session, by saving a record of the settings and items in a session and applying them as far as possible to a different data set.

Predefined evaluation methods for common applications are provided with the system. Custom evaluation methods may be created from user-defined evaluation sessions. An evaluation method is required for evaluation of a data set. Settings and items created by the method can be edited or deleted within the session. An empty method that does not apply any settings or create any items is included with the predefined methods, and may be applied if you do not want to use method-controlled evaluation.

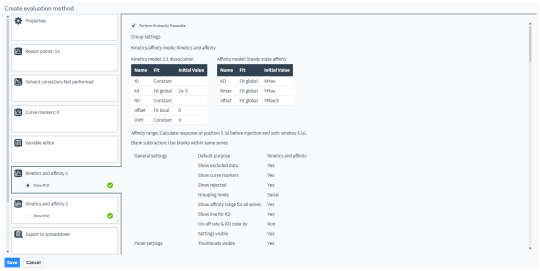
Predefined evaluation methods

Predefined evaluation methods containing settings and items recommended by Cytiva for evaluation of the specific applications are provided with the system. The predefined methods are stored outside the database and cannot be overwritten or deleted.

Creating custom evaluation methods

Custom evaluation methods are created from evaluation sessions, and contain settings and evaluation items present in the session.

Step	Action
1	Open or create an evaluation that contains all settings you want to include in the method. For example, if you want to create a method that involves solvent correction, you must have an evaluation applying solvent correction. ***Note:*** *Settings for* ***Export to spreadsheet, Export to presentation*** *and* ***Export to PDF*** *must be defined in advance, if they are to be included in the method.*
2	Go to the evaluation ***Home*** workspace and select ***Create evaluation method***.

Step	Action
	Result: The workspace lists all items that will be included in the evaluation method. Items with a green checkmark are optional. Remove the checkmark to exclude an item from in the method. 
3	Select a setting or item in the left panel to show details in the right. In some cases, the detail panel may include options (see *Scope of evaluation methods, on page 282*). If the evaluation includes kinetics and affinity items using more than one fitting model, select the model to be included in the method. The evaluation method may not contain more than one model for each of kinetics and affinity. Settings for ***Initial values*** are included with the model saved in the evaluation method. ***Note:*** *The **Sensorgram type settings** in **Properties** overwrites settings from the run. Including them in evaluation methods should be done with care for 1 series systems, since 1 series flow cell usage may vary between runs to a great extent.*
4	Check ***Show first*** for the item you wish to be displayed first when the method is applied. ***Note:*** *This setting does not affect the order in which items are created.*
5	Select ***Save*** and provide a name and location for the method.

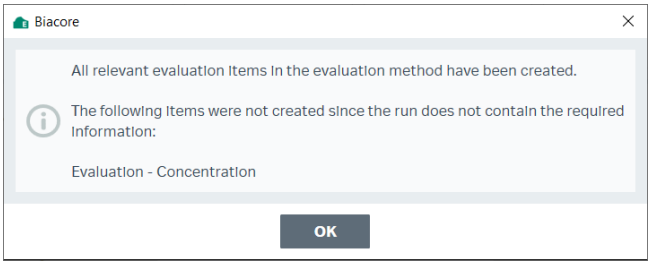
Previewing evaluation methods

Follow the steps below to preview the contents of an evaluation method. You can preview an evaluation method without closing the current evaluation.

Step	Action
1	Select ***Create new evaluation*** if necessary to go to the database view.
2	Either select a run (in order to open the ***Select evaluation method*** tab) or search for evaluation methods. Navigate to the required method.
3	Choose  ***Preview*** from the ***Action*** column. If the icon is not visible, choose ***Show more columns***.
4	Select a component in the method to view the component details.

Applying evaluation methods

Evaluation methods are applied when data sets are opened (see *Section 2.2 Opening runs for evaluation, on page 24*). Components in the methods will be applied as far as possible to the data set. A report detailing which components could not be applied is displayed when the method application is complete.



If you want to apply a different evaluation method to an open data set, you must close the data set and re-open it with the new method.

Scope of evaluation methods

Evaluation methods can include most settings and items in the evaluation session. Important exceptions are listed below:

- Some settings in ***Select sensorgrams***
- Sensorgram markers
- Experiment-specific actions such as excluding sensorgrams or points.
- Filtering individual samples or cycles

For evaluation methods that include kinetics and affinity items, the fitting model(s) in the method will be applied to all groups when the method is used. If the option ***Perform fit directly if possible*** is checked in the method definition, fitting will be attempted for all groups.

12 Action history

Introduction

The ***Action history*** is a read-only log of all actions saved in the database, presented chronologically. It is available from the control software as well as the evaluation software. The ***GxP*** software extension opens more functionality, refer to *Biacore Insight GxP User Manual (29312548)*.

Click a row to display more details about the action, if available.

Sort and filter

The ***Action history*** quickly becomes extensive upon frequent Biacore usage, but there are several tools for finding specific information.

Action	Instruction
Filter	Click ***Filter*** next to a header name and choose what to show. Some information is found in sub-menus or by searching a name. Click ***Apply***. Multiple columns can be filtered simultaneously. Active filters are indicated by a blue filter symbol. ***Tip:*** *Unselect **All** to quickly reduce the number of presented actions.* ***Note:*** *Chip id are sorted based on the date they were first docked.*
Clear all filters	Open the ***Filter*** dropdown menu and click ***Clear all***.
Save a filter	Open the ***Filter*** dropdown menu. Enter a name for the current filter settings and click ***Save***. A maximum of 10 filters can be saved for each user profile.
Apply a saved filter	Open the ***Filter*** dropdown menu and select a filter from the list. Both pre-defined and user-defined filters are available.
Remove a saved filter	Open the ***Filter*** dropdown and click ***Remove*** next to the saved filter. ***Note:*** *Pre-defined filters cannot be removed.*

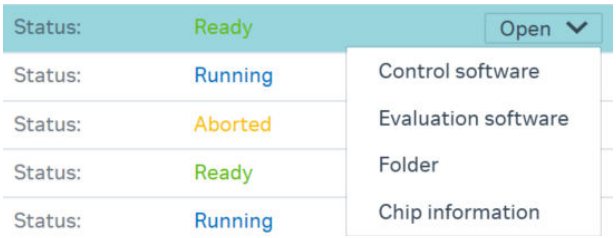
Action	Instruction
Change number of visible actions	Set a number in the ***Show ... items*** drop-down list. Use the page selection buttons to step through the log.
Sort on date	Click on the heading of the date column to toggle between oldest or newest first.
See new actions	Click ***Refresh***. New actions can appear.
Export information	Click ***Print*** to save a PDF in landscape format. The current filter settings apply.

Open action data

Additional action data, such as methods, run data, evaluations, and chip information, can be accessed from the drop-down menu furthest to the right, if applicable. See image below.

Available options vary depending on action type and can open result windows or transfer you to related areas within Biacore Insight Control Software or Biacore Insight Evaluation Software.

Double-click anywhere on an action row to open the first item in the action data drop-down menu.



13 Support for regulated environments

GxP compliance

GxP is a general abbreviation for "good practice" quality guidelines and regulations including GLP (Good Laboratory Practice), GMP (Good Manufacturing Practice), and GCP (Good Clinical Practice).

Support for using Biacore Insight Evaluation in GxP contexts is provided by the ***GxP*** extension, licensed separately from the main software. Functionality is described in detail in the separate *29312548 Biacore Insight GxP User Manual*.

The ***GxP*** extension affects some of the regular workspaces and may, depending on user role, also show the workspaces ***Procedure management***, ***Signatures***, and ***Users*** in Biacore Insight Evaluation Software.

Appendix A
Curve fitting procedures

About this appendix

This appendix describes the principles and models used for fitting curves to data points in Biacore Insight Evaluation software.

In this chapter

A.1 Principles of curve fitting

Introduction

Both kinetics and affinity are evaluated by fitting a mathematical model of the interaction to the experimental data. It is important to realize that fitting is a purely mathematical procedure, that does not have any "knowledge" of the biological significance of parameters in the model equations. Obtaining a good fit is not in itself evidence that the model describes the physical reality of the interaction. Any mechanistic conclusions drawn for the interaction from fitting results (e.g., concerning multiple interaction sites or conformational changes) should be tested using independent techniques.

In other instances of curve fitting such as 4-parameter fitting and relative potency estimation, a mathematical equation derived from theoretical principles is fitted to the data points.

Fitting procedure for kinetics and affinity

Kinetic and affinity parameters are extracted from experimental data by an iterative process that finds the best fit for a set of equations describing the interaction. The fitting process begins with initial values for the parameters in the equations, and optimizes the parameter values according to an algorithm that minimizes the sum of the squared residuals.

In some situations, the fitting algorithm may be unable to find a fit for the experimental data with the initial parameter values as specified in the model. Occasionally, it may be necessary to adjust the initial values for fitting parameters.

Local and global parameters

Parameters in the fitting equations are treated as *local variables*, *global variables*, or *constants* as described in the following table.

Parameter type	Description
Local variables	Assigned an independent value for each curve in the data series.[1]
Global variables	Assigned one single value that applies to the whole data series.
Constants	Assigned a fixed value that is not changed in the fitting procedure.

[1] For single-cycle kinetics (see *Section 9.1 Experimental formats, on page 209*), setting the bulk refractive index parameter RI to local assigns an independent RI value to each sample injection. All other local parameters are assigned a value for each sensorgram.

Evaluating kinetics or affinity with global rate constants gives a more robust value for the rate constants, although the curves may fit the experimental data more closely if all parameters are fitted locally. This is because local fitting allows variation between the constants obtained from different curves: when the constants are fitted globally, this variation appears in the closeness of fit rather than the reported values. Rate constants are always global in predefined kinetic models.

In general, rate constants should be fitted as global parameters and bulk refractive index contribution as a local parameter. The analyte binding capacity of the surface R_{max} is normally set to global, but may be evaluated as a local parameter if there is reason to believe that the surface capacity may vary between cycles in serial mode or channels in parallel mode.

The local/global parameter status is not relevant for affinity determination, since this evaluation fits the model to a single curve of response against analyte concentration.

A.2 Fitting models for kinetics and affinity

Introduction

A set of predefined models for kinetics and steady state affinity is provided with Biacore Insight Evaluation software.

Mass transfer in kinetic models

All kinetic models except ***1:1 dissociation*** include a term for mass transfer of analyte to the surface. If transfer is slow compared with binding of analyte to the ligand, the transport process will limit the observed binding rate, at least partially. Rate constants can be extracted from partially mass transfer limited data.

Mass transfer can be described in terms of transfer of analyte (A) between bulk solution and the surface, with the same rate constant in both directions. Only analyte at the surface can interact with ligand (B). As an example, the simple 1:1 interaction scheme may be represented as

$$A_{bulk} \underset{k_m}{\overset{k_m}{\rightleftharpoons}} A_{surface} + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

Mass transfer parameters

The rate of mass transfer of analyte to the surface under the conditions of non-turbulent laminar flow that prevail in the flow cell is characterized by the *mass transfer coefficient* k_m (units $m \cdot s^{-1}$):

$$k_m = 0.98\left(\frac{D^2 \cdot f}{0.3 \cdot h^2 \cdot w \cdot l}\right)^{1/3}$$

Parameter	Description
D	Diffusion coefficient of the analyte ($m^2 \cdot s^{-1}$)
f	Volume flow rate of solution through the flow cell ($m^3 \cdot s^{-1}$)
h, w, l	Flow cell dimensions (height, width, length in m)

The mass transfer coefficient can be normalized for molecular weight and adjusted approximately for the conversion of surface concentration to RU, to give a parameter referred to as the mass transfer constant k_t (units $RU \cdot M^{-1} \cdot s^{-1}$):

$$k_t = k_m \times MW \times G$$

where G is the conversion factor from surface concentration to RU. The value of G is approximately 10^9 for proteins on Sensor Chip CM5.

A further modification of this expression gives the flow rate-independent component of the mass transfer constant (units RU · $M^{-1}s^{-2/3}m^{-1}$), referred to as t_c. It is this parameter that is reported in kinetic analysis with Biacore Insight Evaluation software.

$$t_c = \frac{k_t}{\sqrt[3]{f}}$$

1:1 binding

This is the simplest model for kinetic evaluation, and is recommended as default unless there is good experimental reason to choose a different model. The model describes a 1:1 interaction at the surface:

$$A + B = AB$$

Default initial values for the ***1:1 binding*** model are listed below.

Parameter	Description	Fit	Value
ka	Association rate constant ($M^{-1}s^{-1}$)	Global	1e5
kd	Dissociation rate constant (s^{-1})	Global	1e-3
Rmax	Analyte binding capacity of the surface (RU)	Global	Ymax
tc	Flow rate-independent component of the mass transfer constant	Global	1e8
RI	Bulk refractive index contribution	Constant	0
Drift	Linear drift (RU/s)	Constant	0

1:1 dissociation

This model fits the dissociation phase of the sensorgrams to an equation for exponential decay, representing dissociation of a homogeneous 1:1 complex. The fitting is independent of analyte concentration. The equation includes an offset term to allow for a non-zero residual response after completion of the dissociation.

$$R = R_0 e^{-k_d(t - t_0)} + \text{Drift} \times (t - t_0) + \text{Offset}$$

Default initial values for the ***1:1 dissociation*** model are listed below.

Parameter	Description	Fit	Value
kd	Dissociation rate constant (s^{-1})	Global	1e-3
offset	Residual response above baseline after complete dissociation (RU)	Local	0
Drift	Linear drift (RU/s)	Constant	0

Note: *This model cannot handle initial response changes resulting from bulk refractive index contributions. If the sensorgrams show bulk contributions, remove data ranges so that the fitting starts after the bulk change is complete.*

Note: *This model does not take account of the effect of mass transport limitations.*

Note: *Application of this model can be sensitive to initial parameter settings. If a good fit cannot be obtained with apparently reasonable dissociation data, try adjusting the initial values to correspond more closely with expected results.*

Bivalent analyte

This model describes the binding of a bivalent analyte to immobilized ligand, where one analyte molecule can bind to one or two ligand molecules. The two analyte sites are assumed to be equivalent. The model may be relevant to studies among others with signaling molecules binding to immobilized cell surface receptors (where dimerization of the receptor is common) and to studies using intact antibodies binding to immobilized antigen. As a result of binding of one analyte molecule to two ligand sites, the overall binding is strengthened compared with 1:1 binding. This effect is often referred to as avidity.

A + B = AB

AB + B = ABB

Note: *Once analyte is attached to the ligand through binding at the first site, interaction at the second site does not contribute to the SPR response. For this reason, the association rate constant for the second interaction is reported in units of $RU^{-1}s^{-1}$, and can only be obtained in $M^{-1}s^{-1}$ if a reliable conversion factor between RU and M is available. For the same reason, a value for the overall affinity or avidity constant is not reported.*

Default initial values for the ***Bivalent analyte*** model are listed below.

Parameter	Description	Fit	Value
ka1	Association rate constant for the first site ($M^{-1}s^{-1}$)	Global	1e5

Parameter	Description	Fit	Value
kd1	Dissociation rate constant for the first site (s^{-1})	Global	1e-3
ka2	Association rate constant for the second site ($RU^{-1}s^{-1}$)	Global	1e-3
kd2	Dissociation rate constant for the second site (s^{-1})	Global	1e-3
Rmax	Analyte binding capacity of the surface (RU)	Global	Ymax
tc	Flow rate-independent component of the mass transfer constant	Global	1e8
RI	Bulk refractive index contribution	Constant	0
Drift	Linear drift (RU/s)	Constant	0

Heterogeneous ligand

This model describes an interaction between one analyte and two independent ligands. The binding curve obtained is simply the sum of the two independent reactions. The relative amounts of the two ligands does not have to be known in advance.

A + B1 = AB1

A + B2 = AB2

Note: *The model is limited to two ligands because the fitting algorithm tends to become unstable with more components, and three or more ligand species cannot be reliably resolved.*

Default initial values for the ***Heterogeneous ligand*** model are listed below.

Parameter	Description	Fit	Value
ka1	Association rate constant for the first ligand ($M^{-1}s^{-1}$)	Global	1e5
kd1	Dissociation rate constant for the first ligand (s^{-1})	Global	1e-3
ka2	Association rate constant for the second ligand ($M^{-1}s^{-1}$)	Global	1e5
kd2	Dissociation rate constant for the second ligand (s^{-1})	Global	1e-3

Parameter	Description	Fit	Value
Rmax1	Analyte binding capacity of the first ligand (RU)	Global	Ymax
Rmax2	Analyte binding capacity of the second ligand (RU)	Global	Ymax
tc	Flow rate-independent component of the mass transfer constant	Global	1e8
RI	Bulk refractive index contribution	Constant	0
Drift	Linear drift (RU/s)	Constant	0

Two state reaction

This model describes a 1:1 binding of analyte to immobilized ligand followed by a conformational or other change that stabilizes the complex. To keep the model simple, it is assumed that the changed complex can only dissociate through reversing the conformational change:

$$A + B = AB = AB^*$$

Note: *Conformational changes in ligand or complex do not normally give a response in Biacore systems. A good fit of experimental data to the two-state model should be taken as an indication that conformational properties should be investigated using other techniques (e.g. spectroscopy or NMR), rather than direct evidence that a conformational change is taking place.*

Default initial values for the ***Two state reaction*** model are listed below.

Parameter	Description	Fit	Value
ka1	Association rate constant for analyte binding ($M^{-1}s^{-1}$)	Global	1e5
kd1	Dissociation rate constant for the complex (s^{-1})	Global	1e-2
ka2	Forward rate constant for the stabilizing change (s^{-1})	Global	1e-3
kd2	Reverse rate constant for the stabilizing change (s^{-1})	Global	1e-3
Rmax	Analyte binding capacity of the surface (RU)	Global	Ymax

Parameter	Description	Fit	Value
tc	Flow rate-independent component of the mass transfer constant	Global	1e8
RI	Bulk refractive index contribution	Constant	0
Drift	Linear drift (RU/s)	Constant	0

Steady state affinity

This model calculates the equilibrium dissociation constant K_D for a 1:1 interaction from a plot of steady state binding levels (R_{eq}) against analyte concentration (C). The equation includes an offset term which represents the response at zero analyte concentration.

$$R_{eq} = \frac{CR_{max}}{K_D + C} + offset$$

Note: *Reported K_D values that are higher than half the highest analyte concentration used should be treated with caution. If the response against concentration plot does not flatten out sufficiently because the concentrations are not high enough in relation to the K_D value, the reported value may be unreliable.*

Default initial values for the ***Steady state affinity*** model are listed below.

Parameter	Description	Fit	Value
KD	Equilibrium dissociation constant	Global	Xmax
Rmax	Analyte binding capacity of the surface (RU)	Global	Ymax
offset	Response at zero analyte concentration	Global	Ymax/5

Steady state affinity (constant Rmax)

This model uses the same equation as the simple steady state affinity model, but sets the R_{max} parameter to a constant. The value for R_{max} is obtained for each analyte from a value entered for a control analyte and the relative molecular weights of control and sample. Select ***Adjust Rmax for ligand decay for all series*** to compensate for assay drift using repeated control samples.

$$Rmax_{analyte} = Rmax_{control} \times \frac{MW_{analyte}}{MW_{control}}$$

Evaluation with this model requires the ***Extended Screening*** or ***Biacore Intelligent Analysis*** extension.

Steady state affinity (constant Rmax and multi-site)

This model fits data from interactions that exhibit binding to multiple sites. Two sites are accommodated in the model.

The model uses a constant R_{max} value for one site, defining the expected stoichiometry, and a fitted value for the other site, which can give an apparent value with undefined stoichiometry.

$$R_{eq} = \frac{CR_{max1}}{K_{D1} + C} + \frac{CR_{max2}}{K_{D2} + C} + \text{offset}$$

Select ***Adjust Rmax for ligand decay for all series*** to compensate for assay drift using repeated control samples.

Evaluation with this model requires the ***Extended Screening*** or ***Biacore Intelligent Analysis*** extension.

Default initial values for the ***Steady state affinity (constant Rmax and multi-site)*** model are listed below.

Parameter	Description	Fit	Value
KD	Equilibrium dissociation constant for the main (strong) binding (M)	Global	Xmax
KD2	Equilibrium dissociation constant for the secondary (weak) binding (M)	Global	100*Xmax
Rmax	Analyte binding capacity for the main binding (RU)	Constant	(Input)[1]
Rmax2	Analyte binding capacity for the secondary binding (RU)	Global	Ymax
offset	Response at zero analyte concentration	Global	Ymax/5

[1] The default initial value is set to Constant=Ymax if no input value for R_{max} has been applied.

A.3 Other fitting models

Linear

Linear fitting uses a function with the form ***y = ax + b***, where ***a*** and ***b*** are constants.

Second degree polynomial

Second degree polynomial fitting uses a function with the form $y = ax^2 + bx + c$, where ***a***, ***b***, and ***c*** are constants).

4-parameter and EC_{50}

Four-parameter fitting uses the following equation. EC_{50} determination in ***Plot*** items identifies the parameters with more descriptive names:

$$y = R_{hi} - \frac{R_{hi} - R_{lo}}{1 + \left(\frac{x}{A_1}\right)^{A_2}}$$

Parameter	Description	EC_{50} name
x, ***y***	Plot coordinates	-
R_{hi}	Fitted parameter corresponding to the maximum y-value	Upper asymptote
R_{lo}	Fitted parameter corresponding to the minimum y-value	Lower asymptote
A_1	x-value at the inflection point of the fitted curve	EC50
A_2	Slope of the fitted curve at the inflection point.	Hill coefficient

PLA

PLA estimates relative potency by fitting the linear region of the sample and reference plots to a model with common slope and individual intercepts.

The common slope β is determined by fitting to the equation:

$$\beta = \frac{\sum_{1}^{n_R}(x_{iR} - \bar{x}_R)(y_{iR} - \bar{y}_R) + \sum_{1}^{n_T}(x_{iT} - \bar{x}_T)(y_{iT} - \bar{y}_T)}{\sum_{1}^{n_R}(x_{iR} - \bar{x}_R)^2 + \sum_{1}^{n_T}(x_{iT} - \bar{x}_T)^2}$$

Parameter	Description
β	Slope of the linear region
x, y	Log(conc) and response values respectively
R	Values for the reference substance
T	Values for the test substance

Relative potency is calculated as:

$$\text{Relative potency} = \bar{x}_R - \bar{x}_T - \frac{\bar{y}_R - \bar{y}_T}{\beta}$$

Confidence intervals for PLA and EC_{50}

Confidence intervals for PLA are calculated in accordance with Fieller's theorem.

Confidence intervals for EC_{50} are calculated according to standard practice in the field.

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