Biacore 1 series

User Manual

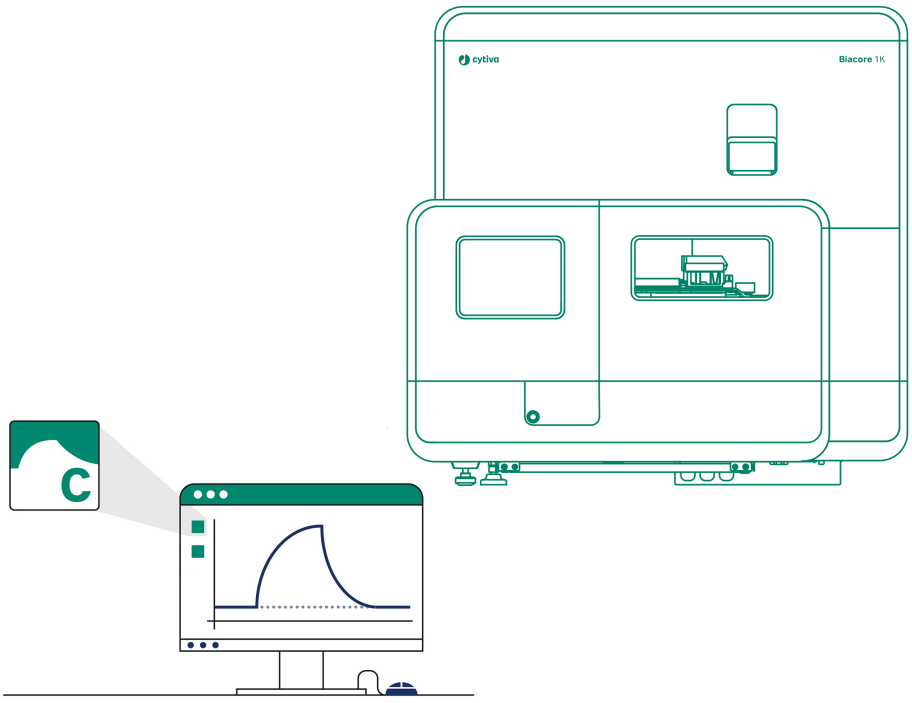

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Table of Contents

1 Introduction

About this chapter

This chapter contains information about this manual and associated user documentation, important user information and intended use of the product. It also contains an introduction to the terminology used for Biacore™ systems.

In this chapter

1.1 About this manual

Purpose of this manual

The *Biacore 1 series User Manual* describes how to use the instrument to perform label-free interaction analysis experiments. Evaluation of the data obtained is described in the separate *Biacore Insight Evaluation Software Manual*.

Scope of this manual

Instrument descriptions in this User Manual apply to Biacore 1K systems, Biacore 1K+ systems and Biacore 1S+ systems. The three systems together are referred to as the Biacore 1 series. Unless otherwise explicitly stated, information in this User Manual applies to all three systems.

Biacore Insight Control Software also controls the Biacore 8 series, comprising of Biacore 8K and Biacore 8K+. For Biacore 8 series, refer to the *Biacore 8 series User Manual*.

Descriptions of the instrument control software apply to version 5.0 or later of the Biacore Insight Control Software.

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.*

1.2 Important user information

Read this before operating the product

All users must read the entire *Operating Instructions* before installing, operating or maintaining the product.

Always keep the *Operating Instructions* at hand when operating the product.

Do not operate the product in any other way than described in the user documentation. If you do, you may be exposed to hazards that can lead to personal injury and you may cause damage to the equipment.

Intended use

The Biacore 1 system, consisting of the Biacore 1 instrument, Biacore Insight Control Software, and Biacore Insight Evaluation Software, supports execution and evaluation of label-free interaction analyses based on surface plasmon resonance (SPR) measurements. The system is intended for research use and quality control measurements associated with manufacturing procedures. The system may not be used for any clinical or diagnostic applications.

Prerequisites

In order to operate the Biacore 1 series in a safe way and in accordance with the intended purpose the following prerequisites must be met:

- The system should be installed according to the instructions in the *Installation* chapter of the *Operating Instructions*.
- You should have a general understanding of the use of a personal computer running Microsoft® Windows® in the version provided with your product.
- You should be acquainted with the use of general laboratory equipment and with the handling of biological materials.

A system administrator familiar with management of Microsoft SQL Server® databases is required. Familiarity with database management is not required for operation of the Biacore 1 series.

Safety notices

This user documentation contains safety notices (WARNING, CAUTION, and NOTICE) concerning the safe use of the product. See definitions below.

WARNING

WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.

CAUTION

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.

NOTICE

NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

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.*

1.3 Associated documentation

Introduction

This section describes the user documentation that is delivered with the product, and how to find related literature that can be downloaded or ordered from Cytiva.

User documentation

The main components of the documentation for the Biacore 1 series are listed in the table below. For more information about applications and general aspects of Biacore, refer to Biacore Application Guides and Biacore online learning.

Translations of the *Biacore 1 series Operating Instructions* are provided in PDF format on the documentation CD inside the back cover of the printed *Operating Instructions*. Other documentation and training material are available from *cytiva.com/biacore*.

Documentation	Main contents
Biacore 1 series Operating Instructions 29706295	Instructions needed to install, operate and maintain the Biacore 1 series in a safe way. In the Biacore 1 series documentation, this will be referred to as the *Operating Instructions*.
Biacore 1 series User Manual 29706293 (this manual)	Detailed system description and instructions for preparing and running experiments. In the Biacore 1 series documentation, this will be referred to as the *User Manual*.
Biacore Insight Evaluation Software User Manual 29287248	Detailed instructions for using the Biacore Insight Evaluation Software to evaluate the results of experiments with the Biacore 1 instrument.
Software help	On-screen assistance for using the Biacore Insight Control Software and Biacore Insight Evaluation Software.
Biacore 1 series Site Preparation Guide 29706296	Requirements for space, power and other supplies, and environmental conditions for installing and running the Biacore 1 series. Required for system installation.
eLicensing Guide for Biacore Systems 29287250	Instructions for handling electronic software licenses. Required for system installation.

Documentation	Main contents
Biacore Insight Database Installation and Management Guide 29287249	Instructions for installing and maintaining the database used to store data from the Biacore 1 series. Intended for the database administrator.

User documentation on the web

Links to laboratory guidelines, application notes, documentation and other online resources may be found on *cytiva.com/bcappsupport*. You will need to register on the web site to access some of these links.

1.4 Glossary

Biacore terminology

Terms used in work with Biacore systems are explained in the following table.

Term	Meaning
Absolute response	The magnitude of the SPR signal measured from the detector baseline.
Active surface	The sensor surface in the flow cell used for analysis of the interaction.
Adjustment for controls	Adjustment of the sample response for changes in the surface activity during the course of an experiment, by normalizing with reference to control sample responses measured at intervals.
Analysis cycle	A sequence of injections of liquid over the sensor surface, 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.*
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.
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.

Term	Meaning
Capture	The term ***capture*** is used to refer to 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.
Detection spot	The area on the sensor surface where detection occurs. In the Biacore 1 series, there is one detection spot in each flow cell.
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.
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 where detection occurs. The flow system of the Biacore 1 series includes six flow cells arranged in series.
Immobilization	The term ***immobilization*** is used to refer to 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.*
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).

Term	Meaning
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	Median response over a short window (typically 5 s).
Running buffer	Buffer used for continuous flow during an experiment.
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 plot of response against time during one analysis cycle. Normally, ***sensorgram*** refers to a single plot from either a single flow cell (reference or active) or the difference between flow cells (reference-subtracted).
Serial flow	A flow pattern where the same liquid flows through two or more flow cells, one after another. In the Biacore 1 series, the flow cells can be addressed in series or separately.
SPR	Surface plasmon resonance, the detection principle used in Biacore instruments.

1.5 Data storage and management

Introduction

All data from the Biacore 1 series (method definitions, runs, and evaluations) is stored in a Microsoft SQL Server database. Installation of a network database is strongly recommended. A local database with limited capacity running on SQL Server Express may be installed on the system computer, but is recommended for service use only. Instructions for installing and managing the database are given in the separate *Biacore Insight Database Installation and Management Guide*.

Data stored on a network is accessible to all users of the Biacore Insight software according to their membership in database roles. Data stored locally may be accessible from other computers depending on local IT policies.

The Biacore 1 series can be set up with multiple separate databases. You select the database to be used when you log in to the software.

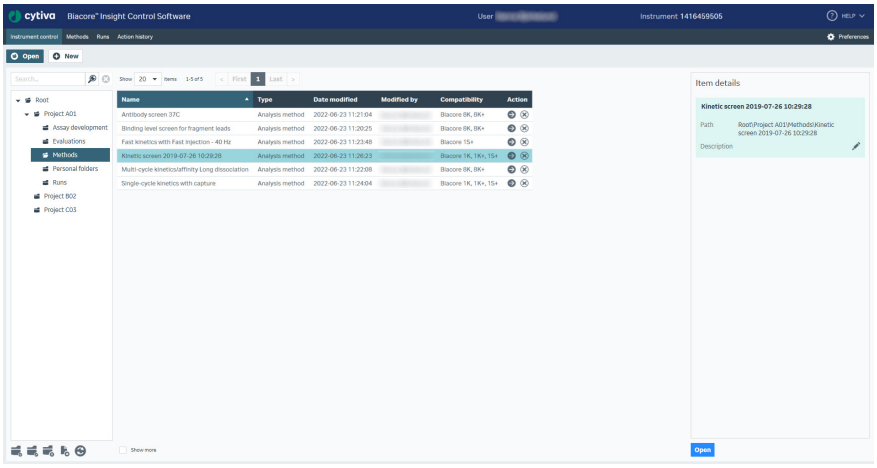
Note: *If multiple users work on the same database object at the same time, the user who first saves changes can save the object with the original name. Other users must save their changes as a new object, using* ***Save as****.*

Data organization

Data is organized in folders in the database. User data (methods, runs, evaluation methods, and evaluations, collectively referred to as *database objects*) are stored in user folders, under a top-level ***Assay*** folder. Storing user data directly in the ***Assay*** folder is not recommended.

The folder structure is displayed in the left-hand panel, with the contents of the selected folder in the right-hand panel.

Tip: *To find a folder easily, select any row in the left-hand panel and type the first letter(s) of the folder. Type again to go to next.*



Note: *All folders and objects in the database are accessible to all users. See the Biacore Insight Database Installation and Management Guide for security management options.*

Opening database objects

To open a database item in the Biacore Insight Control Software, double-click the item in the workspace or select the item and click ***Open***. Methods and runs are opened in the ***Methods*** and ***Runs*** workspaces respectively. See *Section 6.1 Managing methods, on page 70* and *Section 7.1 Opening a run, on page 109* for more details.

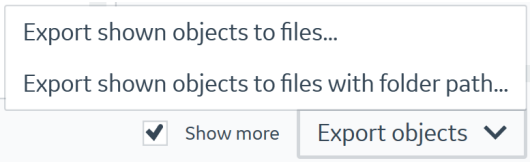
Runs imported from Biacore T200 and Biacore S200 systems cannot be opened in the Biacore Insight Control Software.

Evaluations cannot be opened in the Biacore Insight Control Software.

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 right-hand panel of the workspace to a different folder in the left-hand panel.
Rename object	Click twice on the object name in the right-hand 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 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*** at the bottom 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. 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.*

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.
Import		Use this function to import files to the Biacore Insight database.
Refresh		Refresh the display.

Searching for database objects

Follow the steps below to search for objects in the database.

Step	Action
1	Enter a search term in the ***Search*** field. Enclose phrases containing spaces in single or double quotation marks to search for the entire phrase (e.g., searching for ***new method*** will find ***new*** and ***method***, but searching for ***"new method"*** will find only the complete phrase ***new method***). Search terms are not case-sensitive.
2	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 as described in the table below.

Category	Parameter	Description
Search for [1]	***Runs***	Finds runs with the search term in the name.
	Run methods	Finds immobilization and analysis methods with the search term in the name.

Category	Parameter	Description
	Evaluations	Finds evaluations with the search term in the name. ***Note:*** *Evaluations and evaluation methods cannot be opened in Biacore Insight Control Software.*
	Evaluation methods	Finds evaluation methods with the search term in the name.
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.
Filter on	***Date***	Finds objects modified in the specified date range.
	Users	Finds objects modified by the specified user(s).
	Instrument id	Finds results and evaluations containing data from the selected instrument(s). Select the instrument id 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.*

Category	Parameter	Description
	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.

[1] At least one category must be selected.

2 System description

About this chapter

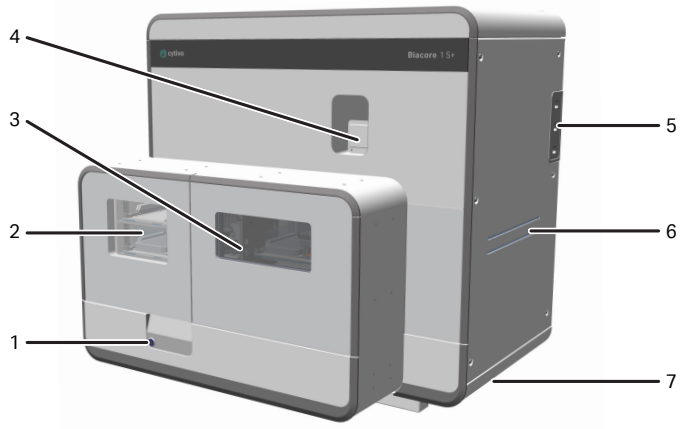
This chapter describes the Biacore 1 series.

In this chapter

2.1 Instrument components

Overview

The main parts of the Biacore 1 series are identified in the illustration below.



Part	Function
1	Hotel door release button
2	Sample hotel door with window
3	Sample compartment with window
4	Sensor chip port
5	Tubing panel
6	Rail for accessory holders
7	Hand grips for lifting (not visible in illustration)

Sample hotel and sample compartment

The sample hotel is the area where microplates and reagent racks carrying samples and reagents can be inserted and removed to a tray by the user. There are two tray positions in the Biacore 1K+ and Biacore 1S+ instruments, referred to as upper and lower, and one tray in the Biacore 1K instrument,

The sample compartment holds one tray at a time. Trays are moved from the hotel to the sample compartment by an automatic sample loading mechanism as required. The sample compartment is the same in Biacore 1K, Biacore 1K+, and Biacore 1S+.

The sample hotel and sample compartment are maintained at the same nominal temperature, set in the Control Software (see *Section 2.3 Temperature control, on page 28*).

Sample hotel door

The sample hotel door can be opened at any time except when a sample tray is being transferred between the hotel and the sample compartment.

Open the sample hotel door by pressing the release button, or by selecting the ***Open*** function in the ***Instrument control*** workspace in the Control Software.

Close the sample hotel door by closing it gently until the magnetic lock engages.

NOTICE

Do not leave the sample hotel door open unnecessarily, as this affects the temperature regulation of the sample compartment and sample hotel.

Hotel door release button

Illumination on the sample hotel door release button indicates the status as follows:

Illumination	Status
Steady	The button is active. Pressing the button opens the hotel door.
Off	The button is inactive (because of tray transfer between the hotel and the sample compartment). Pressing the button has no effect.
Flashing	The hotel door is open.

Sample illumination

The illumination in the sample hotel and sample compartment can be switched on and off from the Control Software. Switch the illumination off if your samples are light-sensitive.

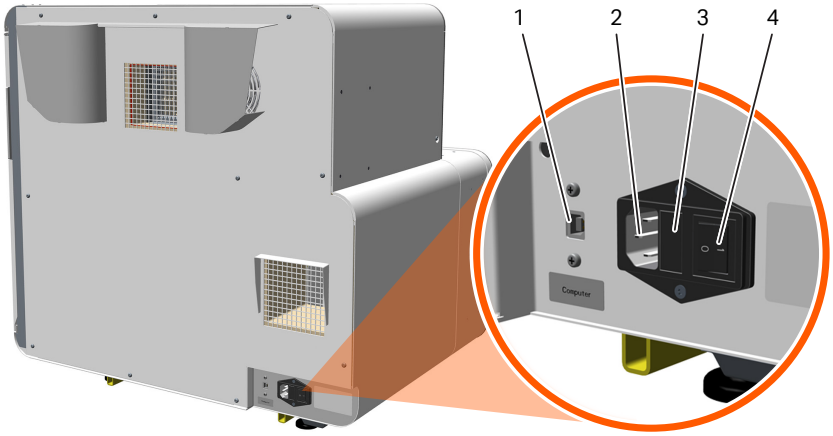
Sensor chip port

The sensor chip port is controlled from the software and cannot be opened by hand. See *Insert a sensor chip, on page 37* for further details.

Electrical connections

The electrical connection panel is located at the lower rear of the instrument on the left-hand side.

The illustration below shows the electrical connections for the Biacore 1 series.



Part	Function
1	USB connector (for connection to controlling computer)
2	Mains power connector
3	Mains fuses
4	Mains power switch

2.2 Flow system

Liquid supply

Running buffer and distilled water are supplied from bottles placed on the bench at the right of the instrument. The Biacore 1K instrument has one buffer connected, while the Biacore 1K+ and Biacore 1S+ instruments can have up to four different buffers connected. Smaller bottles and tubes (up to 1000 mL) may be placed in accessory holders attached to the holder rail.

Glass bottles with caps for buffer and water are provided with the instrument. Any laboratory bottles with screw caps may be used. Bottle caps must be perforated for inlet tubing, and should be vented to prevent accumulation of over- or underpressure as the volume of liquid changes. Suitable caps are provided with the instrument.

Liquid filtering requirement

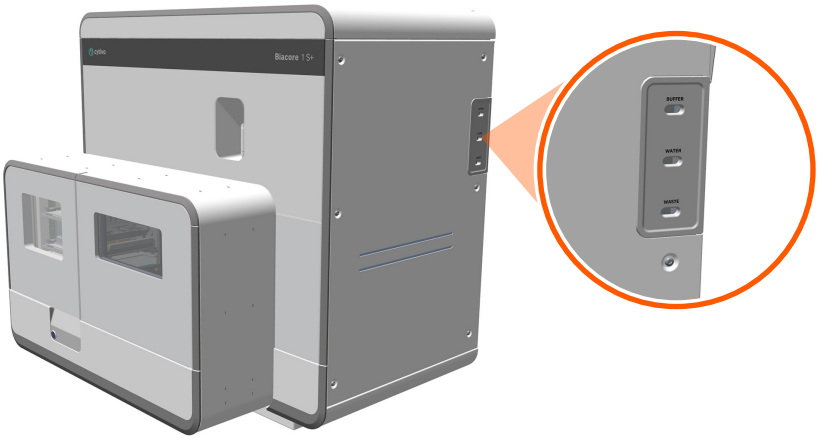
All buffers must be filtered through a 0.22 µm filter to avoid introducing unwanted particles into the flow system. Particles can lead to disturbances in the SPR response, and cause blockage or other malfunction to the microfluidic system.

Tubing inlet panel

Liquids are pumped in to the flow system through inlet tubes on the right-hand side of the instrument. The tube ports are labeled **BUFFER** for running buffer, and **WATER** for distilled water. The Biacore 1K+ instrument and Biacore 1S+ instrument have 4 buffer inlet tubes, labeled **BUFFER A** to **BUFFER D**, providing support for up to 4 different running buffers. Buffer inlets are selectable from the Control Software. Biacore 1K has one buffer inlet.

The **BUFFER** (Biacore 1K)/**BUFFER A** (Biacore 1K+ and Biacore 1S+) and **WATER** tubes should be supplied with liquid at all times during instrument operation, including standby.

The illustration below shows the tubing inlet panel.



Buffer selector

The Biacore 1K+ and Biacore 1S+ instruments are fitted with a buffer selector that allows running buffer to be switched automatically between activities in the activity queue. Up to four buffers are supported. Buffer cannot be switched within an activity in the activity queue.

The Biacore 1K instrument has a single inlet for running buffer and does not support buffer selector functions.

Continuous flow pumps

Continuous flow of liquid (running buffer or sample) over the sensor chip surface is managed by two high precision syringe pumps, housed inside the instrument. The syringe pumps are not accessible to the user.

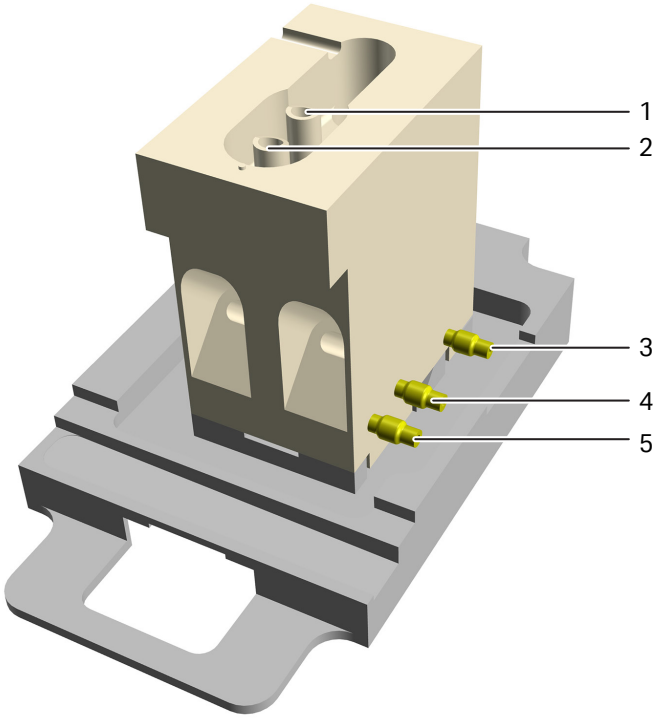
Peristaltic pumps

Three peristaltic pumps provide a supply of running buffer and water for washing the needle during a run. The peristaltic pumps also pump the effluent from the liquid supply block to waste.

The peristaltic pumps are not accessible to the user.

Liquid supply block

Buffer and water used for automatic needle wash are supplied to the injection needle through the ***liquid supply block*** in the sample compartment.



Part	Function
1	Buffer supply
2	Water supply
3	Waste outlet port
4	Buffer inlet port
5	Water inlet port

Integrated microfluidic cartridge (IFC)

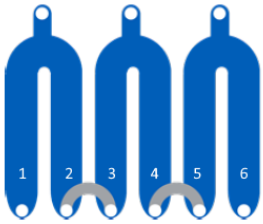
The IFC (Integrated microfluidic cartridge) consists of a series of micro-channels and membrane valves encased in a plastic housing, and serves to control delivery of liquid from the liquid supply block to the sensor chip surface. Grooves on the IFC surface that come into contact with the sensor chip form the flow cells when the sensor chip is docked in the instrument.

Flow cells

The flow cells can be addressed individually as single flow cells, in pairs, in quadruples (only Biacore 1K+ and Biacore 1S+) or all together (only Biacore 1K+ and Biacore 1S+), as presented in the table below.

	Biacore 1K	Biacore 1K+ and Biacore 1S+
single	1	1
	2	2
	3	3
	4	4
	5	5
	6	6
pair	1, 2	1, 2
	3, 4	3, 4
	5, 6	5, 6
quadruple	N/A	1, 2, 3, 4
		3, 4, 5, 6
all	N/A	1, 2, 3, 4, 5, 6

The illustration below shows a schematic representation of the six flow cells through the channel flow path. The wider blue paths have contact with the sensor surface. The white circles are inlets and outlets. The grey paths connect the flow cells.



Waste drainage

The waste tubes exit from the instrument at the right-hand side. Waste drains from the flow system through the waste tubes to either a glass waste bottle placed at the right-hand side of the instrument, or a waste collector funnel mounted on the right-hand side of the instrument to which additional waste tubing can be connected. The latter enables collection in a larger container beneath the instrument. A 2 L waste bottle with cap, the waste collector funnel and an additional tube are provided.

An additional drainage tube collects condensation and any spillage or leakage from the sample compartment, and drains to underneath the instrument. Condensed water that drains from the sample compartment will normally evaporate, although condensation volumes may be significant if the instrument is used in a humid atmosphere, particularly with low temperatures in the sample compartment.

Note: *The volume of liquid draining to underneath the instrument should normally be insignificant. Volumes may increase during operation at low sample compartment temperatures in a humid atmosphere. If significant volumes are observed under other circumstances, check the sample compartment for leaks or blockage in the waste drainage from the liquid supply block.*

2.3 Temperature control

Flow cell temperature

The temperature at the sensor chip is referred to as the ***flow cell temperature***. SPR response is highly sensitive to temperature, and precise control of the flow cell temperature is essential for reliable performance.

The flow cell temperature is for the Biacore 1K and Biacore 1K+ instruments controlled within the range 25°C to 37°C, and for the Biacore 1S+ instrument within the range 4°C to 40°C (cooling possible to at least 18°C below ambient for Biacore 1S+). The temperature is set in the control software, and runs do not start (unless explicitly allowed to do so) if the actual temperature is not equilibrated to the set value.

Sample compartment temperature

The sample compartment temperature is for the Biacore 1K and Biacore 1K+ instruments controlled within the range 4°C to 37°C, and for the Biacore 1S+ instrument within the range 4°C to 40°C (cooling possible to at least 18°C below ambient for all three instruments). The sample compartment temperature is controlled with lower precision than the flow cell temperature, and does not affect the ability to start a run.

If the sample compartment temperature differs from the flow cell temperature, injected solutions equilibrate to the flow cell temperature during transfer from the microplate or reagent rack to the flow cells. However, for maximum performance (particularly at high flow rates), the sample compartment temperature should be set to the same value as the flow cell temperature.

Sample hotel temperature

In the Biacore 1 series, the sample hotel is actively maintained at the same temperature as the sample compartment. For optimal temperature regulation, keep the sample hotel door closed except when handling sample trays in the hotel.

2.4 Sample handling

Sample trays

Samples and reagents are placed on a sample tray, either in a microplate or in a vial in a reagent rack. Each sample tray can hold one microplate and one reagent rack. The Biacore 1K+ instrument and the Biacore 1S+ instrument have two sample trays, one on the upper position and one on the lower position inside the instrument.

The microplates and reagent racks are mounted on the sample tray inside the instrument before they are loaded into the sample compartment. The arrows on the sample tray show how to move the locking levers when releasing a microplate or reagent rack, see the illustration below. The **A1** markings show how to orient the microplate and reagent rack.

Microplates

Samples and low volume reagents are placed in 96- or 384-well standard or deep-well microplates. Microplate specifications and recommendations can be found in the ***Related Documents*** tab of the system product page at *cytiva.com/biacore*. Using microplates of other models or brands may impair the system.

Note: *Do not use polystyrene microplates with samples that contain DMSO.*

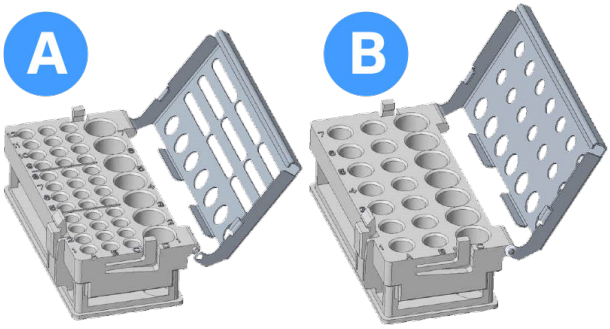
Reagent racks

Reagent rack type **A** can hold 36 vials with 7 mm in diameter (BR100212), and 7 glass or plastic vials (Ø 16 and 15 mm, BR100209 and 29266981), see the left hand illustration below.

Reagent rack type **B** can hold 14 vials with 11 mm in diameter (BR100287), and 7 glass or plastic vials (Ø 16 and 15 mm, BR100209 and 29266981), see the right hand illustration below.

All vials should be covered with penetrable caps.

Close the reagent rack lid before placing it on the sample tray. Use the button on the side to open it.



Foil and septa

Cover microplates immediately after preparation to prevent evaporation. The cover is penetrated by the injection needle when solution is taken from the wells. Adhesive foil and septa are available from Cytiva.

- Use foil where solution is taken only once from each well or vial.
- Use septa where solution is taken more than once from any well or vial.

NOTICE

Place the foil or septa carefully so that the openings are free of adhesive. Adhesive that sticks to the injection needle can seriously impair system performance.

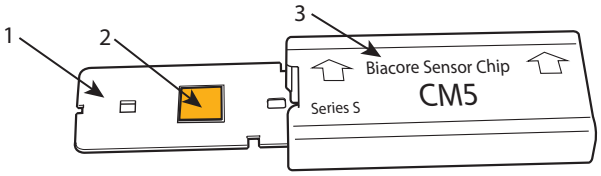
Sample injection

Samples are aspirated from a sample tray and injected over the sensor chip surface by the needle in the sample compartment. Switching between sample and running buffer during analysis is controlled by micro-valves in the IFC (see *Integrated microfluidic cartridge (IFC), on page 25*).

2.5 Signal detection and processing

Sensor chip

The sensor chip is a gold-coated glass slide mounted on a supporting frame, enclosed in a protective cassette. Do not remove the sensor chip from the cassette. The illustration below shows the sensor chip separated from the cassette for illustration purposes.



Part	Function
1	Frame
2	Gold-coated glass slide
3	Cassette

Surface plasmon resonance (SPR)

Interactions are monitored through ***surface plasmon resonance (SPR)*** (surface plasmon resonance) in the gold film on the sensor chip surface. SPR occurs under conditions of total internal reflection of incident light on the glass side of the gold film, and leads to a reduction in the intensity of reflected light at a specific combination of wavelength and angle of reflection (the ***SPR angle***). The wavelength is fixed in Biacore systems. The SPR angle is sensitive to the local refractive index of solution very close (within about 150 nm) to the sensor surface on the opposite side of the gold film, so that changes in the SPR angle can be used to monitor the changes in concentration at the sensor surface as interaction proceeds. The light used to generate the signal does not pass through the sample.

SPR response data

The SPR response is monitored continuously in real time by a 2-dimensional detector array that measures the SPR angle for each detection spot. The response is expressed in ***resonance units (resonance units, RU)***. As a rough approximation for proteins on Sensor Chip CM5, 1 RU corresponds to a change in surface concentration of 1 pg/mm^2. This correlation can differ for different molecules and on different sensor chip surfaces.

The raw SPR response is processed and buffered by a microprocessor in the instrument itself, before being transferred to the external computer for display and storage. This configuration means that real-time monitoring continues even when the processing capacity of the external computer is temporarily interrupted. The time resolution of the measurement can be set to 1, 10, or 40 Hz (40 Hz is only possible with Biacore 1S+).

3 Basic operation and strategies

About this chapter

This chapter describes the basic operation of the Biacore 1 series together with essential considerations for the design and execution of experiments using the system.

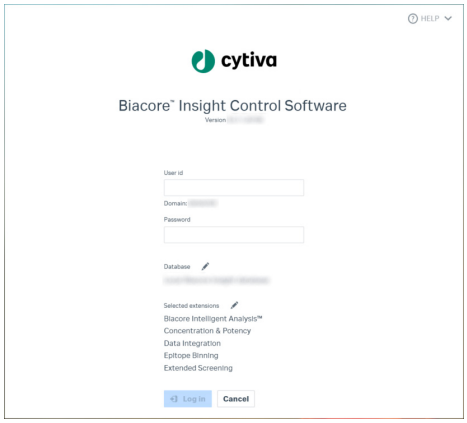
In this chapter

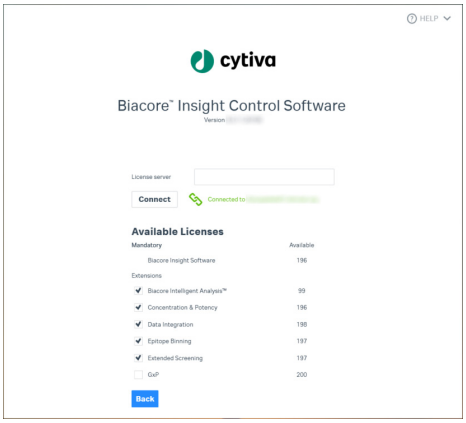
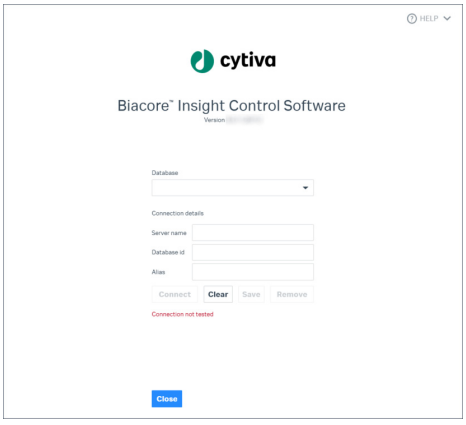
3.1 Starting the system

If the system is switched off

Follow the steps below to start the system and Biacore Insight Control Software:

Step	Action
1	Switch on the power to the instrument (see *Electrical connections, on page 22*).
2	Start the computer.
3	Start Biacore Insight Control Software. *Result:* The login dialog is displayed.



Step	Action
4	Make sure that the correct license server and software extensions are selected. If a warning symbol beside the ***Selected extensions*** list is present, no connection to the license server is specified or the connection has been lost. Click the pen icon to specify the server details and to select extensions.  Enter the license server name and click ***Connect***. Contact your system administrator if you need assistance. Select the software extensions you wish to use. The number of available licenses for each extension is shown in the dialog. Click ***Close*** when you have entered the details.
5	Click the pen icon beside the ***Database name*** to change the selected database. Contact your system administrator if you are uncertain. 

Step	Action
6	Enter your account credentials. Your Windows user name and password are valid as credentials for Biacore Insight software. ***Note:*** *Account credentials do not have to be the same for login to Windows and the Biacore Insight software, provided that valid credentials are used in both cases.* ***Note:*** *Biacore Insight software does not support Windows Fast User Switching.*
7	Click ***Log in***.
8	Wait until the instrument self-test is completed and connection to the computer is established as indicated by the instrument number at the top of the screen.

No instrument connected

If a connection to the instrument cannot be established, make sure that:

- The USB cable is properly connected to the computer and the instrument.
- The instrument is switched on.

If a connection still cannot be established, contact your service representative.

If the system is in standby mode

If the system is in standby mode (the instrument status panel at the bottom of the screen shows ***Running standby flow***), no further action is needed. Standby mode will be stopped automatically when a new instrument activity is started.

3.2 Preparing for a run

Set the temperature

Set the flow cell temperature and sample compartment temperature well in advance of starting the run, to allow time for the system to equilibrate. Equilibration time for a temperature change of 5°C is about 40 minutes.

Methods will not start if the flow cell temperature has not reached the set value. (Methods will however start if the sample compartment temperature has not reached the set value.)

Follow the steps below to set the temperature in the flow cell and/or sample compartment.

Step	Action
1	From the ***Instrument control*** workspace, add ***Set flow cell temperature*** or ***Set sample compartment temperature*** to the activity queue.
2	Enter the required temperature in the activity workspace.
3	Select ***Set temperature***. Temperature equilibration will start immediately if there are no prior activities in the queue. The current temperature and target temperature are presented in the panel at the bottom of the ***Instrument control*** workspace. ***Note:*** *The **Set ... temperature** activities are executed and removed from the queue quickly. However, temperature equilibration normally takes a longer time.*

Set up the liquid supply

Follow the steps below to provide running buffer, distilled water, and waste bottle for the flow system. Make sure that all bottles and waste containers are sealed and standing on spill trays. Two spill trays are included with the system.

Required volumes of running buffer and water for a run are shown in the ***Instrument control*** workspace. Volumes shown are minimum requirements, calculated from estimated consumption plus a dead volume in the bottle.

Note: *Volumes shown do not include requirements for queued activities that start automatically or for standby flow following the run. Add the appropriate volumes of buffer and water to those shown in the workspace (see Standby mode, on page 42 for volumes required during standby).*

Step	Action
1	Fill a bottle with distilled water and place it on the right-hand side of the instrument. Insert the tube marked **WATER**.
2	Fill up to 4 bottles with running buffer(s) as required and place them on the right-hand side of the instrument. Insert the buffer inlet tube(s) according to the buffer requirements for the run. Configuration of the buffer selector, available with Biacore 1K+ and Biacore 1S+, is described in *System setup tools, on page 60*. ***Note:*** *The* **BUFFER** *(Biacore 1K) or* **BUFFER A** *(Biacore 1K+ and Biacore 1S+) tube must always be placed in buffer, even when another buffer is used as running buffer. Other unused buffer tubes are preferably capped or placed in water.*
3	Make sure that all liquid supply tubes are securely placed at the bottom of the liquid.
4	Place the waste container on the right-hand side of the instrument and insert the waste tube, or, mount the waste collector funnel on the right-hand side of the instrument and connect appropriate waste tubing. The tube can be connected to a waste container beneath the instrument. ***Note:*** *There must always be free passage in the waste tube to prevent flooding of the system. Make sure that the tube is not kinked.* ***Note:*** *The height of the waste connection is important to prevent the siphon effect. The capped waste flask must be placed at the same height as the instrument, for example on the same trolley. If instead a waste connector funnel is used, make sure that it is mounted on the rail.*

Insert a sensor chip

If the instrument is in standby mode, a sensor chip will be docked in the instrument. Normally, you will need to replace this sensor chip with one appropriate for your run. Follow the steps below to change the sensor chip. Follow the same steps but omit step 2 if the instrument has been restarted from shutdown.

Step	Action
1	Add ***Change chip*** to the activity queue.
2	If a sensor chip is already docked in the instrument, click ***Undock chip*** and remove the chip from the sensor chip port.

Step	Action
3	Select ***New chip*** and enter the details of the sensor chip for the run. Alternatively, select ***Used chip*** and choose a chip from the list. Only chips from the same instrument series as the connected instrument is visible in the list.
4	Insert the sensor chip and close the chip door.
5	Click ***Dock chip***. ***Note:*** *If you click **Dock chip** before closing the chip door, a notification will be issued. Close the door and select **Retry** in the notification.*

Note: *For runs requiring highest performance, allow the flow system to equilibrate in standby mode at least overnight after changing the sensor chip or changing solutions. Extend the equilibration time to at least 24 h if the detergent concentration in the running buffer is changed.*

Select or create a method

Follow the instructions below to set up the method for the run. See *Chapter 6 Methods workspace, on page 69* for more details.

Step	Action
1	Go to the ***Methods*** workspace in the Control Software.
2	Open a predefined or existing method from the database. Make any modifications that may be required to the method definition.
3	Go to the ***Variables and positioning*** tab. Enter or modify the variable details as required including sample information, microplate types and position assignments.
4	(Optional) Go to the ***Cycle overview*** tab and check that the run is set up correctly. If you need to make any adjustments, return to the ***Method Definition*** tab and adjust the necessary settings.
5	(Optional) Print the ***Plate layout*** information as an aid in preparing microplates.

3.3 Preparing and loading samples

Prepare a microplate

Follow the steps below to prepare samples and reagents in a microplate.

Step	Action
1	Dispense the samples and reagents into the microplate wells according to the ***Plate layout*** information in the method. Make sure that there are no air bubbles trapped at the bottom of the microplate wells. It is particularly easy to trap air bubbles in 384-well microplates. Use of a microplate centrifuge to remove air bubbles is recommended.
2	Cover the microplates as recommended (see *Foil and septa, on page 30*) to prevent evaporation from the samples during analysis. ***Note:*** *Microplate foils and septa can cover the well position identifiers on the microplates. You may want to mark the* **A1** *corner of the microplate after attaching the foil or septum. Take care not to mark the foil or septum directly over a well position.*

Prepare a reagent rack

Follow the steps below to prepare a reagent rack of the same type as defined in the method, **A** or **B**.

Step	Action
1	Press the button on the side of the reagent rack to open the lid.
2	Populate the reagent rack with vials.
3	Dispense the samples and reagents into the vials according to the ***Plate layout*** information in the method. Make sure that there are no air bubbles trapped at the bottom of the vials.
4	Put on penetrable caps on all vials.
5	Close the lid of the reagent rack.

Mount the microplate and the reagent rack on a sample tray

Follow the steps below to mount the microplate or the reagent rack on the sample tray.

Step	Action
1	Pull the lever with the arrow sign on the sample tray toward you to open it.
2	Place the microplate or reagent rack on the sample tray with position **A1** at the front left, as marked on the tray. The microplate slot is to the left and the rack tray slot is to the right.
3	Carefully push the plate or reagent rack all the way in until you hear a click. The lever automatically closes around the microplate or reagent rack. ***Note:*** *Correct plate and rack positions are important to ensure that the injection needle is not damaged. Do not move them once they have been pushed all the way in.*

Release the microplate and reagent rack by pulling the locking levers on the sides.

3.4 Performing the run

Start the run

Follow the instructions below to start the run in the software.

Step	Action
1	Click ***Send to queue*** in the method workspace. *Result:* The method is added to the activity queue.
2	Select the running buffer for the method, if the instrument is equipped with a buffer selector (Biacore 1K+ and Biacore 1S+). ***Note:*** *The* **BUFFER** *(Biacore 1K) or* **BUFFER A** *(Biacore 1K+ and Biacore 1S+) tube should always be placed in buffer, even when another buffer is used as running buffer. Unused buffer tubes should be capped or placed in water or buffer.*
3	Assign the microplate(s) and reagent rack(s) to the sample tray(s) in the software.
4	If the sample hotel door is closed, press the open door button on the front panel or select ***Open*** from the instrument status pane in the Control Software.
5	Open the hotel door fully (see *Sample hotel door, on page 21*).
6	Place the sample microplate(s) and reagent rack(s) on the correct tray in the sample hotel as assigned in the software. ***Note:*** *Using incorrect microplates can damage the injection needle.*
7	Close the hotel door. Make sure that the status is shown as ***Closed*** in the software.
8	Click ***Ready to start***.
9	Navigate to the required folder and provide a name for the run. Click ***Save***. *Result:* The run starts as soon as all previously queued activities are complete. Actual run start can be delayed if the flow cell temperature is not stable (see *Flow cell temperature, on page 28*).

You can load microplates and reagent racks onto tray(s) in the sample hotel at any time except when a tray is being moved between the hotel and the sample compartment. If any of the locking levers are opened when a sample tray in the hotel is required by the method, a notification is issued and the run does not continue until the tray is provided.

NOTICE

The system does not detect the size of the microplate on the tray. It is the user's responsibility to make sure that a microplate corresponding to the software settings is mounted on the tray. Using an incorrect microplate can damage the injection needle.

Monitor the run

Sensorgrams generated as the run progresses are displayed in the ***Instrument control*** workspace (see *Section 5.4 Interactive run, on page 64*, and *Section 5.5 Display during a method run, on page 68*).

At the end of the run, the next activity in the queue starts automatically unless user input is required. Otherwise, the system is automatically placed in standby mode.

Clean-up after the run

The following activities should be performed as required after a run:

- Remove any microplates or reagent racks from the sample hotel, and close the tray locking levers.
- Make sure there is sufficient liquid for the intended standby period.
- Empty the waste bottle.

Standby mode

Always leave the system in standby mode unless the instrument is to be shut down. Standby mode uses the current buffer tube.

Tip: *The current buffer tube is highlighted in blue in the status bar in the Control Software.*

Standby mode maintains a continuous low flow of liquid through the flow system. Recommended liquids for standby operation over longer periods are buffer for the **BUFFER** supply tubing and water for **WATER** tubing. Liquid from the **BUFFER** inlet, but not from **WATER**, passes over the sensor surface during standby.

A sensor chip is required in the instrument during standby operation.

The maximum unattended standby period is 7 days. Select ***Restart*** in the instrument status pane to extend the standby period by resetting the timer to 7 days. Make sure there is sufficient liquid supplied to each inlet for the intended standby period. Approximate liquid consumption for each inlet tubing is listed below:

Tubing	Consumption (mL/24 h)	Passes over sensor surface
BUFFER	130	Yes
WATER	95	No

3.5 Basic strategies

Introduction

The Biacore 1 series offers considerable flexibility for experimental design. The following general consideration can affect the design of experiments and should be kept in mind:

- The same running buffer is used throughout a method, although buffer conditions for sample injections can be varied by using the A-B-A command (see *A-B-A command, on page 93*). A different running buffer can be selected at the start of a new method.
- The flow system contains six flow cells in series, which can be addressed individually, in pairs, in quadruples[1] or all together[1].
- Subtraction of reference from active sensorgrams (reference subtraction within the same cycle) is performed using data from two flow cells. Biacore 1K+ and Biacore 1S+ can assign multiple references per active flow cell, resulting in one sensorgram for each active-reference flow cell pair. Subtraction can only be done with reference flow cells upstream of the active flow cell.
- Subtraction of sensorgrams from different cycles (usually blank subtraction, performed in the Biacore Insight Evaluation Software) may only be performed within flow cells, or flow cell pairs in case of reference subtraction.

Achieving low levels of immobilized ligand

Experience with amine coupling of some proteins on Sensor Chip CM5 has shown that consistent low immobilization levels are best achieved by maintaining the ligand concentration and ligand contact time in combination with one or both of the following approaches:

- Reduce the activation time of the surface with EDC/NHS. Activation times as short as 30 seconds have proved useful.
- Reduce the proportion of EDC mixed with NHS. Using 20% EDC and 80% NHS can reduce the immobilization level by about 50%.

A predefined surface preparation method for amine coupling that implements both of these approaches is provided with the software. Adjust the activation time and/or proportion of EDC and NHS to suit your requirements.

More details may be found in publications on the website (see *User documentation on the web, on page 9*).

[1] Only for Biacore 1K+ and Biacore 1S+

Assay development

Assay development work frequently involves comparison of ligand and analyte behavior under different conditions. The Biacore 1 series may be used to increase efficiency of assay development either by reducing the number of sensor chips required or by completing assay development in a shorter time.

Assay development principles are described in the *Biacore Application Guides* (available from Cytiva). Some examples of steps supported by predefined analysis and/or evaluation methods in the Biacore 1 series are listed below:

- pH scouting for immobilization conditions
- Binding test using a single-cycle kinetics format
- Interaction characteristics
- Buffer scouting using the ***A-B-A*** injection
- Regeneration scouting

Detailed design of assay development work is outside the scope of this handbook. Supporting material for experimental design may be found on the web (see *User documentation on the web, on page 9*).

Screening applications

To exploit the capacity of Biacore 1K+ and Biacore 1S+ fully in screening applications, different ligands or different ligand densities should be immobilized or captured in flow cells 2-6, with flow cell 1 as reference. By letting the analyte flow through all six flow cells, five analyte-ligand pairs can be analyzed each cycle. For Biacore 1K, up to two flow cells can be addressed in each cycle. Results from different cycles can be grouped in the Biacore Insight Evaluation Software to reflect the intention of the experiment.

Immobilized ligand is inevitably constant between cycles. However, if ligand is captured, the ligand can be varied between cycles. With the A-B-A injection (see *A-B-A command, on page 93*), it is possible to vary the solution conditions between cycles and flow cells, for example, for analysing interactions in the presence of a competitor or cofactor.

Concentration analysis

The ***Concentration & Potency*** extension adds support for determination of analyte concentrations using a calibration curve obtained by analysis of known standard samples.

In direct binding assays (DBA), different concentrations of analyte are injected over a sensor surface with attached or capture ligand. Inhibition in solution assays (ISA) investigate the binding of a macromolecule to an immobilized analyte or analyte analogue in the presence of different concentrations of inhibiting analyte. The surface competition assay is an alternative to ISA, suitable when analyte immobilization presents problems.

Predefined analysis methods are provided for the different experimental setups, including parallel and serial microplate layouts, with and without enhancement injections. Concentration measurements are evaluated using dedicated functionality in the Evaluation Software, described in the *Biacore Insight Evaluation Software Manual*.

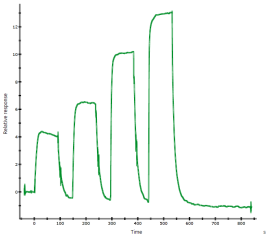
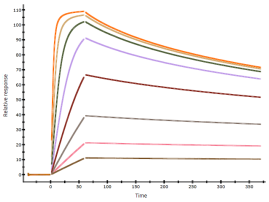
Parallel line analysis and EC_{50} determination

Potency of a pharmaceutical candidate or product can be expressed in terms of EC_{50} (half maximal effective concentration) or in comparison to a reference compound by *PLA* (parallel line analysis, also called parallel line assays).

Support for both PLA and EC_{50} determination is included in the ***Concentration & Potency*** extension.

Kinetics and affinity

Kinetic and affinity determinations both rely on analysis of the interaction over a range of analyte concentrations, and can be approached in several ways, depending on the requirements of the application. Some underlying principles and recommendations are listed in the table below.

Approach	Considerations
Single cycle approach	Increasing analyte concentrations are injected sequentially in the same cycle, with no regeneration between injections. 
Multi-cycle approach	Each analyte concentration is injected in a separate cycle or channel. Regeneration is required between cycles. 
Blank cycles	During evaluation in Biacore Insight Evaluation Software, sensorgrams are corrected by subtraction of blank cycles (analysis cycles with zero analyte concentration). Blank subtraction applies only within flow cells. For single cycle determinations, blank cycles occupy the same number of positions in the microplate as the analyte cycles.

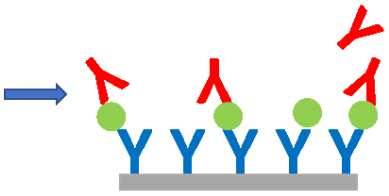
Approach	Considerations
Duplicate cycles	For multi-cycle determinations in serial mode, at least one non-zero analyte concentration may be run in duplicate, with duplicates well separated in the cycle sequence, to provide a check on consistency of surface capacity during analysis. Duplicate analyte concentrations are not relevant to single-cycle determinations.

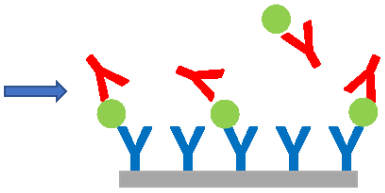
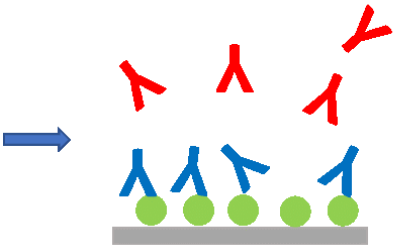
Single- and multi-cycle approaches give fully comparable results.

Epitope binning

Antibodies specific to the same target antigen are tested in a pairwise combinatorial manner to assess whether or not they block one another's binding to an epitope of the antigen. Antibodies that compete for the same epitope and share a common blocking profile are binned together. Predefined analysis methods are provided for the most commonly used assay formats sandwich, premix, and tandem. Epitope binning runs are evaluated using dedicated functionality in the Evaluation Software, described in the *Biacore Insight Evaluation Software Manual*.

Support for epitope binning is included in the ***Epitope binning*** extension.

Assay format	Description
Sandwich	The first antibody is immobilized or captured to the surface. Antigen is then injected over the first antibody, followed by injection of the second antibody. 

Assay format	Description
Premix	The first antibody is immobilized or captured to the surface. The premixed solution with antigen and second antibody is then injected over the first antibody. 
Tandem	The antigen is immobilized to the surface. The first antibody is then injected over the antigen, followed by the second antibody. 

Support for regulated environments

The ***GxP*** extension provides support for work in regulated environments in accordance with the requirements of §21 part 11 of the Federal Code of Regulations. Functionality added by the ***GxP*** extension is described in the separate *Biacore Insight GxP User Manual (29312548)*.

4 Software overview

About this chapter

This chapter describes the general organization of the Biacore Insight Control Software.

The software requires a connection to the instrument in order to control instrument-related operations such as starting and monitoring a run. However, the software can also be used on a computer that is not connected to the instrument, such as an office computer, to create methods and examine runs stored in a network database.

Workspaces

The interface is organized into the following main workspaces:

Workspace	Description	More information
Instrument control	Provides tools for instrument management.	*Chapter 5 Instrument control workspace, on page 52*.
Methods	Used for creating and editing methods.	*Chapter 6 Methods workspace, on page 69*.
Runs	Displays results of runs.	*Chapter 7 Runs workspace, on page 108*.
Action history	Displays a log of instrument and database actions.	*Chapter 8 Action history, on page 117*.

Software extensions

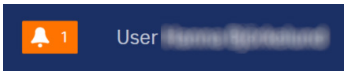
The software for the Biacore 1 series is available as a basic package with optional software extensions. Currently available extensions are listed in the table below.

Extension	Description
Biacore Intelligent Analysis™	Provides support for automatic prediction of results in binding level and affinity screen applications.
Concentration & Potency	Adds predefined method templates and functionality for determination of analyte concentration based on calibration curves.
Data Integration	Provides support for data export in JSON and XML format.

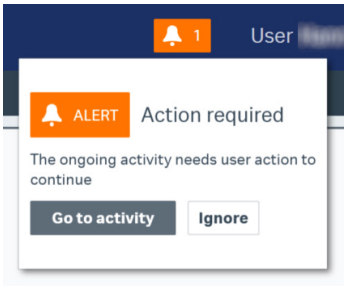
Extension	Description
Epitope Binning	Adds predefined method templates and functionality optimized for epitope characterization.
Extended Screening	Adds predefined method templates and functionality optimized for screening of low molecular weight analytes.
GxP	Provides support for work in regulated environments, 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)*.

Notifications

Alerts and malfunctions are indicated by notifications in the top banner of the screen. The number of notifications is shown in the notification symbol. Notifications are also issued as reminders when scheduled instrument maintenance is due.



Click on the notification symbol to open details of the notification. Most notifications require user action for instrument operation to continue. In some cases, software buttons for user action are shown with the notification text.



Preferences

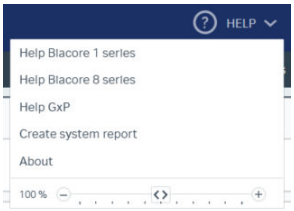
Settings in ***Preferences*** are user-specific and define the instruments accessible in, and supported by, Biacore Insight Control Software. Information and features related to non-selected instruments remains hidden. Changes can only be applied when no method is opened. At least one instrument must be selected to open the rest of the software.

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).

Help

The following functions are provided in the ***Help*** menu:



Function	Description
Help Biacore 1 series/Biacore 8 series	Provides top-level help on the current workspace. Follow the links in the help system to find the information you need.
Help GxP	Provides help about GxP-specific functionality, if the ***GxP*** extension is active.
Create system report	Use this function to generate a report containing information on the software installation and environment, and on the status of the connected instrument (if any). The report is intended to assist Cytiva service representatives in troubleshooting system errors.
About	Provides information about the current software version, the connected database, and the selected extensions.
 Zoom slider	Use the slider to zoom the display in or out. ***Note:*** *Zooming in to the display may obscure some elements of the interface.*

5 Instrument control workspace

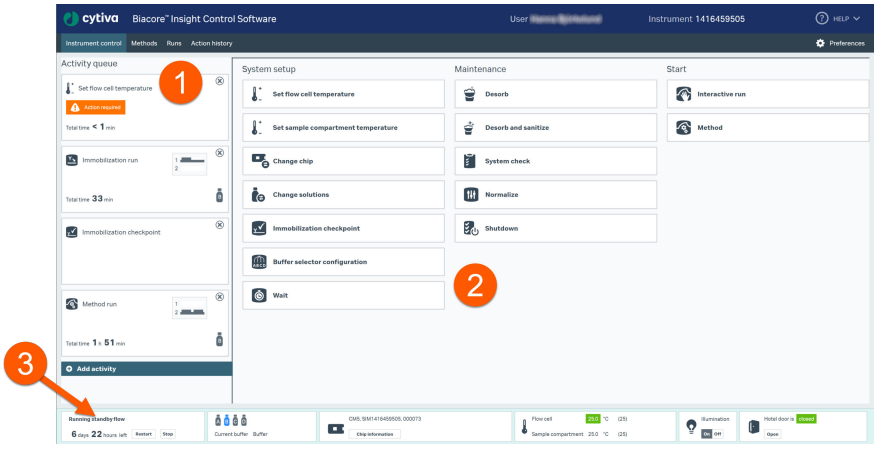
About this chapter

This chapter describes the functions and use of the ***Instrument control*** workspace.

In this chapter

Introduction

Operation of the Biacore 1 series is managed from the ***Instrument control*** workspace. The workspace is divided into three main areas, which are only visible when an instrument is connected. The area borders are fixed.

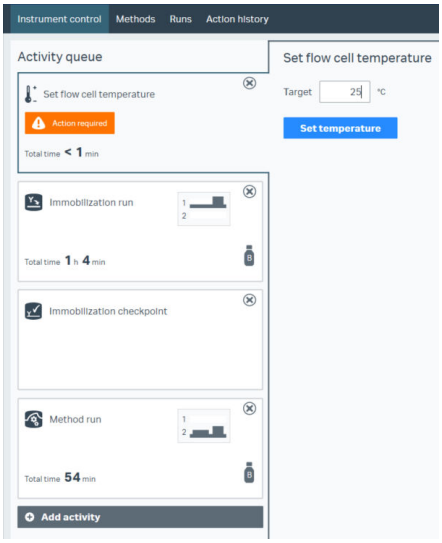


Part	Function
1	Activity queue. Programmed activities (methods and tools) are placed in the queue to be executed on the instrument. See *Section 5.1 Activity queue, on page 54* for details.
2	Main workspace. Shows the available tools when no activity is selected in the queue. Shows the details and any user input requirements for activities selected in the queue.
3	Instrument status. Provides information on the current status of the instrument to which the software is connected, and holds some direct action controls for instrument operation such as opening the sample hotel door and turning the sample compartment illumination on or off.

5.1 Activity queue

Introduction

Operations on the Biacore 1 series are initiated through the ***Activity queue***, displayed in the left-hand panel of the ***Instrument control*** workspace.



An activity that is added to the queue will start automatically when required user input has been provided and all previously queued activities are completed. Conditions that prevent a run from starting are highlighted in orange.

Activities are executed in order from top to bottom. When an activity is completed, the item is automatically removed from the queue.

Adding activities to the queue

To place a tool from the ***Instrument control*** workspace in the queue, simply click the required tool button. If the workspace displays the activity details for an activity already in the queue, select ***Add activity*** in the ***Activity queue*** panel to return to the tool selection display.

Methods are added to the queue from the ***Methods*** workspace (see *Section 6.1 Managing methods, on page 70*).

Activities are added to the bottom of the queue, and can be moved by dragging with the mouse. An activity cannot be moved to a position before the one that is currently being executed.

User input

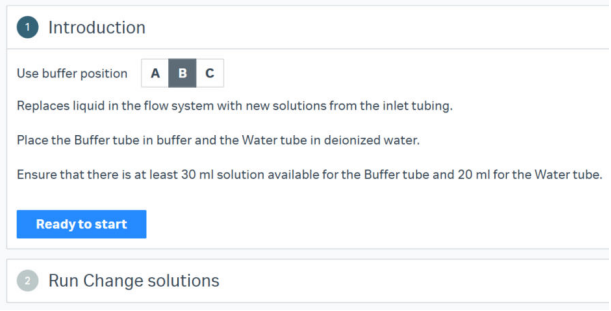
All activities require some kind of user input before they will start. This may be simple confirmation that the activity is ready to start, or more detailed input in the form of parameter values, settings, and tray positions. User input is provided in the main workspace that is displayed when the activity is selected in the queue (and when the activity is first added to the queue).

User input for queued activities can be provided in advance while activities earlier in the queue are being executed. If all input is provided in advance, the activity will start automatically as soon as the preceding activity has been completed.

Selecting buffer inlet

For instruments equipped with a buffer selector, the buffer inlet can be selected when a method or ***Change solutions*** activity is added to the activity queue. The available inputs are determined by the ***Buffer selector configuration*** (see *System setup tools, on page 60*). The buffer inlet cannot be changed during an ongoing activity.

An example of the user input workspace for ***Change solutions*** with the buffer selector configured for 3 inlets is shown below.



The current buffer inlet (buffer ***B*** in the example above) is selected by default. There is no default selection if the buffer selector configuration is changed to exclude the current inlet.

Note: *The name of running buffer for a method is set in the method, and is independent of the buffer inlet used.*

Estimated time for completion of activities

Each activity entry in the queue shows the estimated time required for completion of the activity. If the selected buffer inlet for an activity differs from the previous setting, the estimated time will include the time required to change the buffer in the system.

Managing the activity queue

Procedures for managing activities in the queue are listed in the table below.

Operation	Procedure
Move an activity	Drag the activity item to the new position with the mouse. You cannot move an activity to a position before the activity currently being executed.
Remove an activity	Select ⊗ ***Remove*** in the activity item, then confirm that you want to remove the activity. Any user input provided for the activity will be lost.
Abort an ongoing activity	Select ⬤ ***Stop*** in the activity item, then choose whether to abort with or without washing the system. New confirmation will be required for all following items in the queue when an activity is aborted.

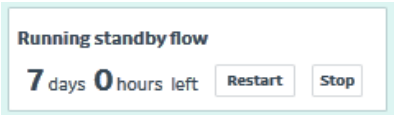
5.2 Instrument status

Overview

The instrument status and other information is shown in the panel at the bottom of the ***Instrument control*** workspace.

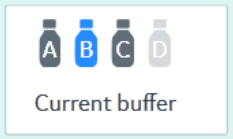
Current instrument activity

This item displays the current instrument activity. When no activity is being executed, buttons for managing standby flow are displayed:



Function	Description
Start standby flow	Displayed when the instrument is idle. Click to start standby flow. Make sure liquid is supplied to all inputs before starting standby flow.
Restart	Displayed while standby flow is running. Click to reset the standby flow timer to 7 days.
Stop	Displayed while standby flow is running. Click to stop standby flow. ***Note:*** *Use this function only if you want to stop standby flow for maintenance purposes. Standby flow stops automatically whenever another activity is started.*

Buffer inlets



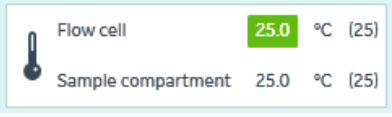
This item shows the current buffer selector configuration and selected buffer inlet. Buffer symbols are colored as follows:

Color	Meaning
B Blue	Currently selected.
C Black	Available but not currently selected.
D Gray	Not included in the current buffer selector configuration.

Sensor chip

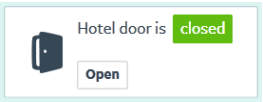
This section displays properties of the currently docked sensor chip. Select ***Chip information*** for more extensive information, including ligand identity and immobilized level in each flow cell.

Temperature



This item displays the current flow cell and sample compartment temperature (with the set values in parentheses). The flow cell temperature is shown in red if the set temperature has not been reached.

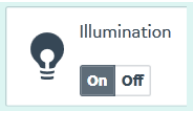
Sample hotel door



This section displays the status of the sample hotel door. Select ***Open*** to release the hotel door lock (see *Sample hotel door, on page 21*).

The door cannot be opened while sample trays are being transferred between the sample compartment and the hotel.

Sample illumination

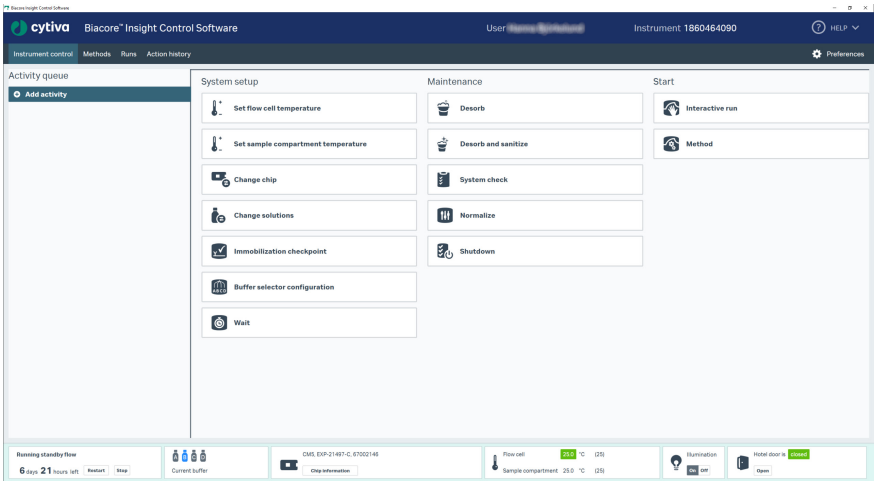


This section displays the status of the sample compartment and hotel illumination. Use the buttons to turn the illumination on and off.

5.3 Instrument control tools

Introduction

The ***Instrument control*** main workspace panel provides access to tools for performing instrument operations and shortcuts to creating a new method or opening an existing method. Click on a tool to add the tool to the activity queue. Click on a method shortcut to go to the ***Methods*** workspace.



System setup tools

Tool	Description
Set flow cell temperature	Sets the temperature at the flow cell.
Set sample compartment temperature	Sets the temperature in the sample compartment. The sample compartment temperature is set independently of the flow cell temperature: however, for best performance, the sample compartment temperature should be the same as or close to the flow cell temperature.
Change chip	Undocks and docks the sensor chip in the instrument. ***Note:*** *For runs requiring highest performance, allow the flow system to equilibrate in standby mode at least overnight after changing the sensor chip.*

Tool	Description
Change solutions	Fills the flow system with solutions. Use this tool when buffer is changed, when the system is started from shutdown, and after docking a chip. Run ***Change solutions*** even when changing to a new batch of nominally the same solution, and to change the selected buffer inlet within the scope of the buffer selector settings. ***Note:*** *For runs requiring highest performance, allow the flow system to equilibrate in standby mode at least overnight after changing solutions. Extend the equilibration time to at least 24 h if the detergent concentration in the running buffer is changed.*
Immobilization checkpoint	Sets an automatic control of the immobilization levels, reducing the need of manual confirmation of adequate immobilization prior to analysis. It compares the chip immobilization levels with acceptance criteria entered by the user. If results are within the acceptance criteria, the activity queue continues with subsequent activities. If any result falls outside the acceptance criteria, the activity queue is paused and user input is required to resume or stop the activity queue. If ***Stop activity queue*** is selected, all subsequent activities are set to ***Action required***. Acceptance criteria can be defined using an upper and/or lower limit and may vary between flow cells.

Tool	Description
Buffer selector configuration	Configures the buffer selector for 1 to 4 buffer inlets and selects the current inlet: • One inlet (**A**) • Two inlets (**AB**) • Three inlets (**ABC**) • Four inlets (**ABCD**) The tool flushes and empties buffer tubes as appropriate in a sequence of steps that depends on the initial and target configurations. Each step requires user input to start. Full instructions are given in the tool. Tubes that are configured as not used should be fitted with protective caps to prevent dust from entering the tubes. Tubing caps are provided with the system. This tool is only available for instruments fitted with a buffer selector (see *Buffer selector, on page 24*).
Wait	Inserts a user-defined delay period into the activity queue. Buffer flow continues during the ***Wait*** period.

Maintenance tools

Tool	Description
Desorb	Cleans the flow system. Run this tool at regular intervals (recommended at least once a week).
Desorb and sanitize	Cleans and disinfects the flow system to prevent growth of microorganisms. Run this tool at regular intervals (recommended at least once a month).
System check	Checks system performance. Run this tool when a malfunction is suspected or when instructed by Cytiva service.

Tool	Description
Normalize	Normalizes the detection system to compensate for small differences in the light distribution in the detection system. ***Normalize*** is recommended to be performed periodically (for example, once a month) as a test of the system, and to fine adjust the detector responses. Normalize should also be used after chip exchange as an extra precaution, if best possible performance is required. The procedure can be run either before a ligand has been attached or before the first analysis run using the immobilized chip.
Shutdown	Empties the flow system and shuts down the instrument. Run this tool when the instrument will be left unused for more than 7 days.

See the *Operating Instructions* for details of maintenance operations.

Start

Tool	Description
Interactive run	Starts an interactive run.
Method	Relocates to the ***Method*** workspace from where a method can be sent to the activity queue.

5.4 Interactive run

Introduction

The ***Interactive run*** workspace lets you take full control of the instrument while providing immediate feedback. In contrast to run methods, cycles are not defined in advance. Instead, you add commands and take decisions based on the result of previous injections, thereby building up the cycle as the run is proceeding.

There are no requirements for when to use ***Interactive run***, but popular applications are:

- Confirmation of surface activity after an immobilization run.
- Quick tests, such as testing whether new analytes can bind, or comparing a small group of analytes.
- Assay development for finding suitable concentration spans, injection times, and regeneration procedures.
- Training and demonstration of the Biacore system.

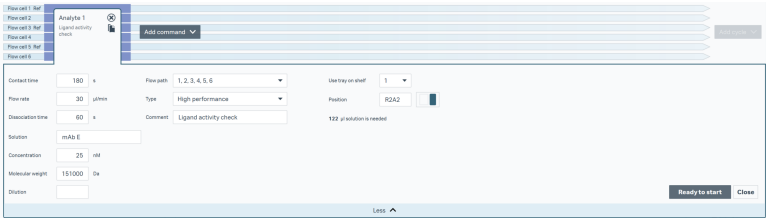
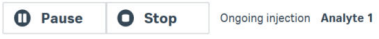
Although technically possible, ligand attachment via ***Interactive run*** is not recommended. By instead going through an immobilization method, adequate wash procedures are included, and the immobilization levels are stored in the chip information. See *Section 6.2 Immobilization methods, on page 76* for more information on available tools to control the level of immobilized ligand within a method.

The commands in ***Interactive run*** are essentially equivalent to the commands of a regular analysis run defined by a method. By including additional details about an interactive run injection, such as the concentration, more advanced evaluation possibilities are enabled in Biacore Insight Evaluation Software. Run methods are however the recommended approach for most standard applications, such as estimation of kinetics, affinity and concentration, to ensure that sufficient data is included.

Perform an interactive run

Follow the steps below to perform an interactive run.

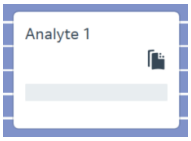
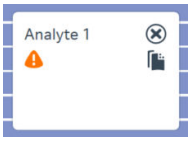
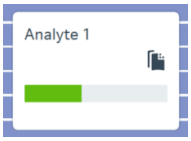
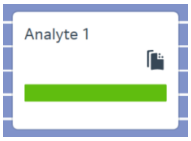
Step	Action
1	Add ***Interactive run*** to the activity queue. *Result:* General run information and settings are displayed.
2	Choose which buffer bottle to use (if the system has a buffer selector), data collection rate, which flow cells to use, if there should be any reference, and the concentration unit of each command.

Step	Action
3	Confirm that ***Tray setup*** is correct or adjust accordingly. ***Note:*** *All trays used during an interactive run must have the same type of microplate and reagent rack.*
4	Click ***Ready to start*** and save the run. *Result:* The ***Activity queue*** panel remains hidden throughout the run.
5	Add a command to the command sequence, enter its required information, and click ***Ready to start***. All injections require information about contact time, flow path, tray and position. The position can either be entered as a coordinate or by selecting a position in the plate view, accessible via the tray icon. ***Note:*** *To add a solution to a tray that is currently in the sample compartment, an* ***Eject tray*** *command must first be executed.* ***Tip:*** *Click* ***More*** *in the settings panel and enter relevant information about the command. The data is stored in the run file and is a convenient way of keeping track of performed operations.*  *Result:* The command will start immediately if there are no unfinished commands in the command sequence. Otherwise, it is put in a pending state until its turn.
6	(Optional) Pause an ongoing injection from the ***Injection pause controls***. Paused injections can either be resumed within a limited time or stopped completely. A few seconds delay and a small consumption of the solution can be expected upon injection pause. 
7	Investigate the results of steps 4-6 using the available tools for zoom, alignment, and response level readout: • Drag around an area to enlarge the region and double-click to zoom out.

Step	Action
	• Move the response ruler to read the response level for selected curves at the given time point, as presented in the sensorgram display and in the ***Response table***. The response level is relative the alignment point, as defined by the alignment ruler. • Click on the curves to select them one by one, or shift to ***Select area mode*** and drag over an area to select all curves partially or wholly within the area. Readouts can be saved together with descriptive names in the ***Response table***. • Move the response ruler to the right end of the x-axis to see the current response for selected curves. For more details, see *Section 7.3 Sensorgram view settings, on page 115*.
8	Repeat steps 5-7 for as many times as you like.
9	(Optional) Divide the run into multiple cycles using the ***Add cycle*** button. Select ***New cycle*** to create an empty cycle, or choose ***Copy cycle*** to include the commands from the previous cycle. User input is required before the commands in the copied cycle can be started. Additional cycles can only be created if at least one command has been executed and there are no ongoing commands. New commands can only be added to the cycle that is currently running.
10	Click ***End run*** or ***Abort run***. Ending an interactive run allows all ongoing and pending commands to finish. If a run is aborted, any commands that are ongoing or not yet executed are stopped or removed from the command sequence. The instrument is put in standby and the ***Instrument control*** workspace becomes accessible. A finished ***Interactive run*** can be opened in the ***Runs*** workspace. ***Note:*** *An interactive run is automatically stopped and saved when a cycle exceeds 72 h (1 Hz), 7.2 h (10 Hz) or 1.8 h (40 Hz).*

Commands

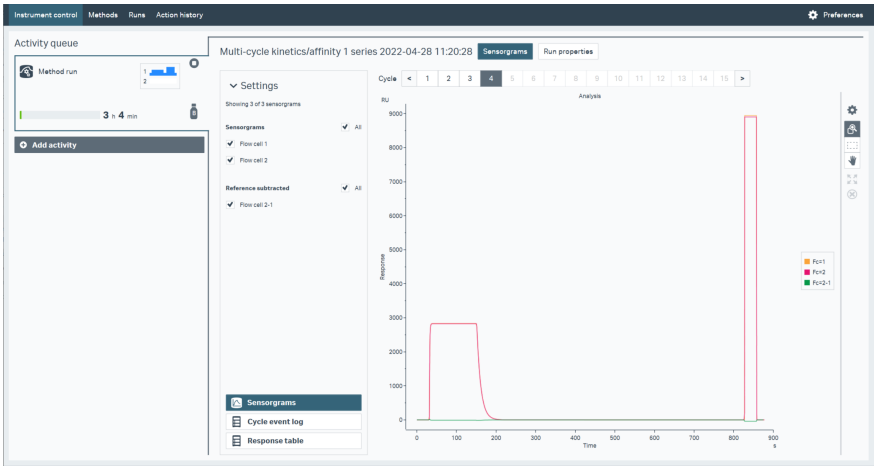
Most of the commands found in run methods are also available in ***Interactive Run*** and behave identically. Manage the command sequence in the same way as the step sequence (see *Managing steps, on page 88*). The status of a command is visualized according to the table below.

Appearance	Description
	The command is ready to start and will be executed when previous commands have finished. It can still be edited and deleted.
	The command awaits user confirmation before it can be started. Select the command, confirm or edit necessary settings, then click ***Ready to start***.
	The command is ongoing. The command can no longer be edited or deleted from the command sequence but can be paused or stopped using the ***Injection pause controls*** if it is an injection.
	The command has finished.

5.5 Display during a method run

Description

During an ongoing method run, the main instrument control workspace panel displays the progress of the sensorgrams from the run. The ***Activity queue*** panel is still accessible. If you select ***New activity*** to add a new activity to the queue, you can return to the run display by selecting the run activity in the queue.



Note: *The illustration above shows sensorgrams from a simulated run.*

The display is the same as that for the ***Runs*** workspace (see *Chapter 7 Runs workspace, on page 108*), except that the activity queue panel is accessible alongside the run display. The activity queue item shows the sample hotel configuration and highlights the sample tray currently in use.

6 Methods workspace

About this chapter

Biacore Insight software supports two kinds of methods. Both kinds are created and edited in the ***Methods*** workspace.

- Immobilization methods, for covalently attaching molecules to the sensor surface.
- Analysis methods, for performing interaction analysis experiments.

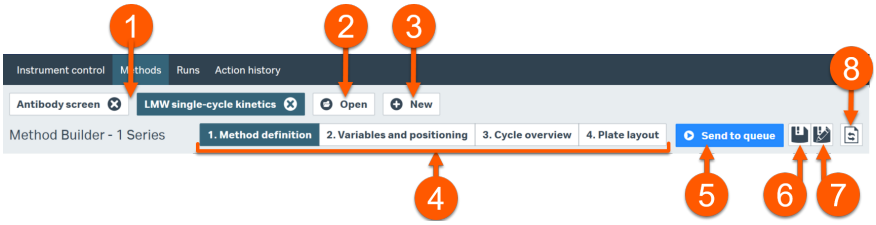
In this chapter

6.1 Managing methods

Introduction

This section describes the ***Methods*** workspace and provides instructions for managing methods. The following sections describe immobilization and analysis methods in detail.

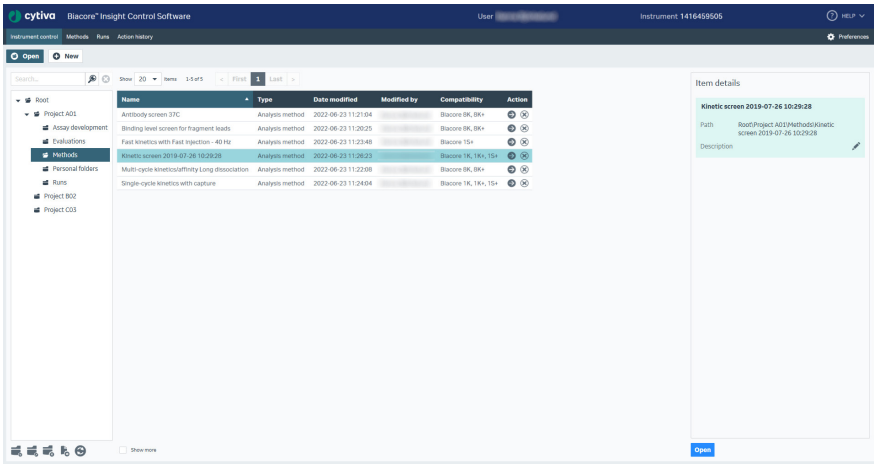
Methods workspace header



Part	Function
1	Tabs for open methods. Multiple methods may be open at the same time. The currently displayed method is highlighted in blue. • Select a tab to make the corresponding method current. • Click ⊗ ***Close*** on a tab to close that method.
2	***Open*** button. Select to open an existing method from the database.
3	***New*** button. Select to create a new method.
4	Method management steps. The steps are different for immobilization and analysis methods.
5	Add the method to the activity queue.
6	Save the method to the database.
7	Save a copy of the method to the database with a new name.
8	Convert the method to be compatible with the other instrument series. Only available in method management step ***1. Method definition***.

Opening a method from the database

Follow the steps below to open a method in the ***Methods*** workspace. A shortcut to this workspace is also provided in the ***Instrument Control*** workspace.



Step	Action
1	Select ***Open*** in the ***Methods*** workspace.
2	Navigate to the required folder or use the ***Search*** function (see *Searching for database objects, on page 16*).
3	Choose the method. Summary details of the chosen method are shown in the panel at the right. ***Note:*** *Only methods compatible with instruments selected in* ***Preferences*** *(see Preferences, on page 50) are visible.*
4	Select ***Open*** or double-click the method. The method will be opened in a new tab.

Opening a method from a run

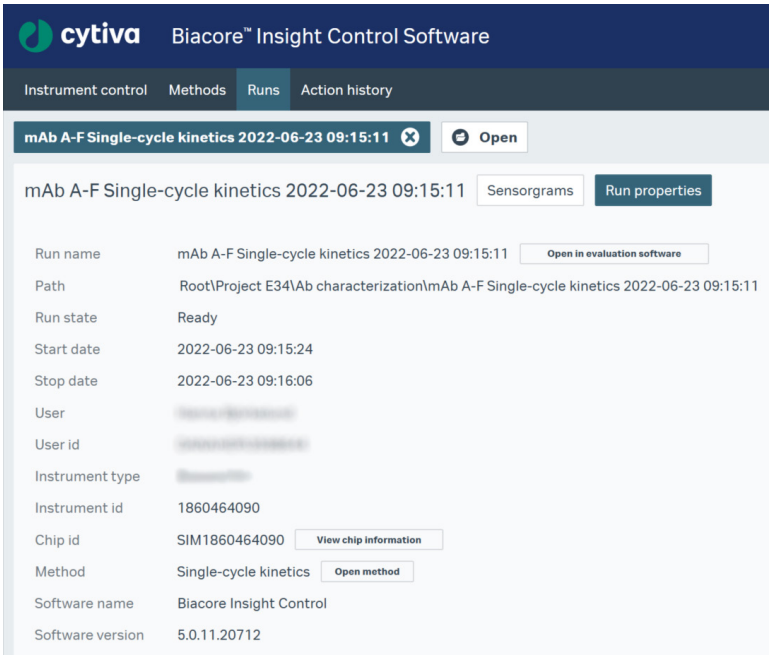
The method definition used for a run is saved with the results of the run, and can be opened from the run item even if the original method item has been changed or deleted. You can edit the method if required and save it with a new name.

Note: *The method definition saved in the run item cannot be changed.*

Follow the steps below to open the method saved in the run item.

Step	Action
1	Open the run (see *Section 7.1 Opening a run, on page 109*).

Step	Action
2	Open the ***Run properties*** tab.



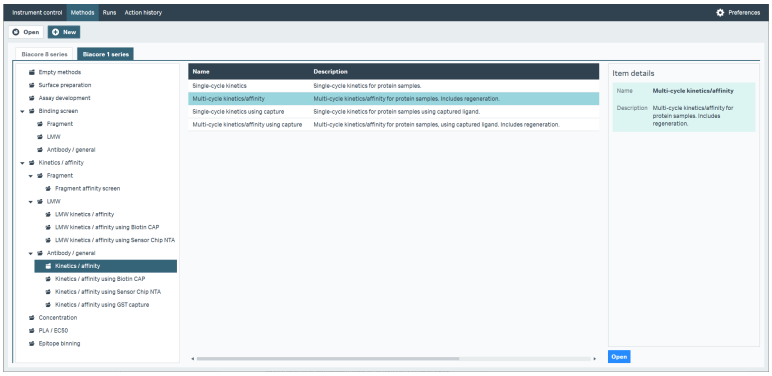
3	Choose ***Open method***. *Result:* The method is opened in a new method workspace, exactly as it was used to perform the run.

Creating a new method

New methods are created using predefined methods as templates. Predefined methods are provided for common application requirements.

Note: *You can also create a new method by editing an existing method and saving it with a new name.*

Follow the steps below to create a new method from the ***Methods*** workspace.

Step	Action
1	Select ***New*** in the ***Methods*** workspace.
2	If instruments of both Biacore 1 series and Biacore 8 series are selected in ***Preferences***, two tabs of pre-defined methods are visible. Choose tab based on the instrument to be used for the run. ***Note:*** *Methods can be converted to be compatible with the other instrument series. See Converting a method, on page 75 for more details.*
3	Select an appropriate predefined method as a template for your method. ***Note:*** *Methods for ligand attachment differ from analysis methods. Immobilization method templates cannot be used to create analysis methods and vice versa.* 
4	Select ***Open*** or double-click on the predefined method. The method template will be opened in a new tab.
5	Edit, save and/or run the method as required. ***Note:*** *Predefined methods cannot be overwritten.*

Editing method definitions

To edit a method, enter new steps, commands and parameter values as required.

See or *Section 6.3 Analysis methods, on page 82* for detailed instructions.

Saving methods

Select ***Save method*** to save the method with the current name. The previous version of the method will be overwritten.

Select ***Save as new method*** to save the method with a new name.

Note: *Predefined method templates cannot be overwritten. If you create a new method based on a template and select* ***Save method*** *you will automatically be redirected to* ***Save as new method****.*

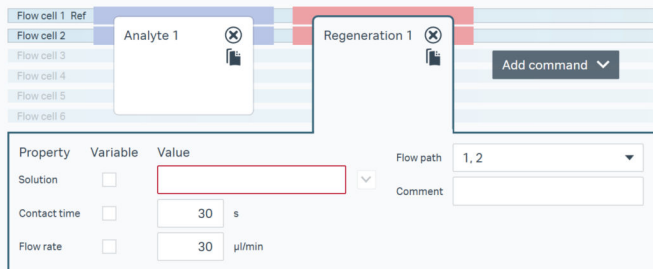
Note: *A copy of the method is automatically saved with the results when the method is run. Methods that have been run but not explicitly saved can be recovered from the results (see* *Opening a method from a run, on page 71**).*

Method verification

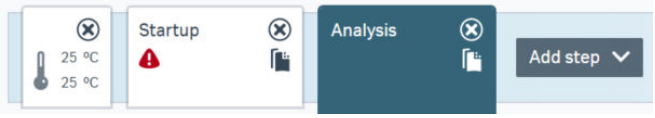
Methods are automatically verified on the fly, to check that steps and commands are correctly specified and that values are provided for all required variables.

Note: *Verification determines only that the method is correctly specified and can be executed. The software cannot check that the method fulfills the intended application purpose.*

Errors are indicated by a red frame around the missing or incorrect information. The example below shows a missing solution in the command ***Regeneration 1***.



Errors in closed steps and commands are indicated by a warning symbol in the respective tab. The example below shows that there are one or more errors in the step ***Startup***.



Note: *The warning symbol is hidden if the step or command is selected. Instead the error indication is visible.*

Compatibility

Compatibility information is displayed in ***Method Builder*** if more than one instrument from the Biacore series of the active method is selected in ***Preferences***. The compatibility is based on method settings and number of samples. A run can only be started if the method is compatible with the connected instrument.

In this example, a Biacore 1 series method is used and Biacore 1K, Biacore 1S+ and Biacore 8K selected in ***Preferences***. The data collection has been set to 40 Hz, which is not supported by Biacore 1K. The circle corresponding to Biacore 1K has turned gray and the cause of incompatibility is presented when hovering over the ***Compatibility*** area. No compatibility information is presented about Biacore 8K since this is not a Biacore 8 series method.

Converting a method

Click ***Convert method*** from ***1. Method definition*** to transform an opened method to fit the other instrument series, i.e. Biacore 1 series to Biacore 8 series or vice versa. Method conversion requires that instruments from both series are selected in ***Preferences***. The converted method can be edited and saved as a new method and is as similar as reasonably possible to its original in terms of method definition. Information from the ***Variables and positioning*** step is not inherited. Settings that are not supported by any of the selected instruments in the other series either require user input or, when this is not possible, are automatically adjusted to become compatible. Flow cell usage and frequency are examples of the latter. Commands not supported by the other series are removed.

Note: *It is not possible to convert immobilization methods.*

Running methods

To run a method, select ***Send to queue*** from the method workspace and provide a name and location for the results. Make sure that the correct chip is docked, the waste bottle is empty, and that buffers and solutions have been prepared and positioned according to the setup. The method will be placed in the activity queue, and will start when the required information has been provided and all preceding activities have been completed.

The ***Send to queue*** button is not available from the ***Method definition*** step.

6.2 Immobilization methods

Introduction

Immobilization methods are used to attach capturing molecules or ligands to the sensor surface. Standard attachment methods on each sensor chip type are supported directly. Custom methods can also be created, either to modify details of standard methods or to create methods for new attachment approaches.

An immobilization method may contain multiple steps with different procedures in different steps, each affecting a selected group of flow cells. Each step results in one cycle when the method is run.

More details of coupling chemistry and attachment methods may be found in the *Biacore Sensor Surface Handbook*.

Note: *Reversible capture of ligands on the sensor surface by binding to an immobilized capturing molecule is not performed with an immobilization method. This step is part of the analysis cycle in an analysis method.*

Setting up standard immobilization methods

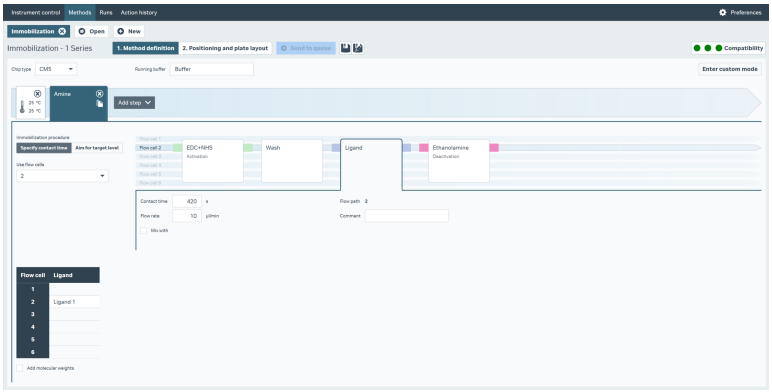
Only the ligand injection conditions can be changed in standard immobilization methods. To change other injection settings, use a custom method (see *Setting up custom immobilization methods, on page 79*).

Follow the steps below to set up a standard immobilization method.

Note: *The immobilization method templates provided with the system are set up for amine coupling. To create a method for a different immobilization chemistry, remove the* ***Amine*** *step and add a step for the appropriate chemistry.*

Step	Action
1	Select ***New*** in the ***Methods*** workspace and choose a suitable predefined immobilization method template from the ***Surface preparation*** folder. Click ***Open***.

Step	Action
	Result: The method opens in a new method tab.

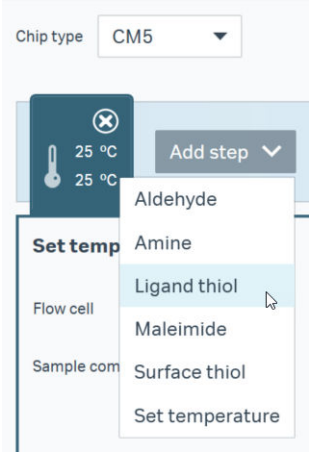


2 Select the ***Chip type*** that you wish to use. The default is ***CM5***. Select ***Custom*** if you are using a chip type that is not listed.

If you select a chip type that is not compatible with steps in the method, you will be prompted to discard the steps.

3 Remove any steps that you do not want from the predefined method and add steps that you require. Each unique ligand must be immobilized in a separate step. Perform steps 4-8 below for each ligand.

To add a step, select ***Add step*** and choose the immobilization chemistry. Only chemistries applicable to the selected ***Chip type*** are listed.



Step	Action
	Note: *Steps added to a method based on the predefined* ***Immobilization low levels*** *method will not include the adaptation to low immobilization levels (see Achieving low levels of immobilized ligand, on page 44).*
4	Choose which flow cells to use for the selected method step. If a pair of flow cells is used, it can be specified which of them that will be exposed to ligand through the ligand command ***Flow path*** setting: • When both flow cells are selected, the ligand will pass serially over both flow cells in a single injection. Immobilization levels can be slightly lower in the latter flow cell. • By addressing the ligand to the last flow cell only, the first flow cell will be blank immobilized. The blank immobilized reference surface is activated and deactivated without exposure of ligand, thereby obtaining charge properties that resemble the ligand surface.
5	Choose between the two immobilization procedures and enter the required values: • Use ***Specify contact time*** to adjust the contact time and flow rate for the ligand injection. The ligand injection can be paused or stopped via the ***Injection pause controls*** before the defined contact time has elapsed, enabling a thorough control of the immobilization level. Paused injections can be resumed within a limited time, potentially increasing the level. Stopping an injection flushes the aspirated liquid to the waste bottle. ***Note:*** *There will be a few seconds delay after selecting* ***Pause*** *before the injection is actually paused.* ***Note:*** *An injection pause consumes a small volume of the solution. The total contact time of a resumed injection will be somewhat shorter than what was specified in the command.* • Use ***Aim for target level*** to let the software control the ligand injection to achieve a specific immobilization level (see *Aim for target level, on page 80*). Enter a target level. When multiple flow cells are in the flow path, the ligand injection stops in all flow cells when the target level is reached in the ***Guiding flow cell***.

Step	Action
6	Select ***Mix with*** to mix the ligand with another solution immediately before injection. Specify the name of the solution to be mixed and the percentage in the final mixture (for example, specifying "90% buffer" will mix 1 part of ligand with 9 parts of buffer). This can be used, for example, to dilute the ligand immediately before immobilization when the ligand shows reduced stability in immobilization buffer. ***Note:*** *Mixing is performed in the reagent rack or microplate and requires an empty vial respectively plate position. The empty vial or position must be on the same tray as the solutions to be mixed.* *Mixing positions are also required for preparing the EDC/NHS mixture used in most immobilization methods.* *Mixing is supported in vials with 7 or 11 mm in diameter, 96-well microplates with well volume 250 or 650 µL, and 384-well microplates with well volume 110 or 200 µL.*
7	Provide ligand name(s) for the selected flow cells.
8	Select ***Add molecular weights*** and enter ligand molecular weights if required. This information is used for calculation of expected binding capacity (R_{max}) values in some evaluation procedures.
9	When the method definition is complete, go to the ***Positioning and plate layout*** step to manage sample and reagent positioning in the microplates and to print positioning information as a guide to microplate preparation.

Setting up custom immobilization methods

Contact time and flow rate are fixed for all injections except the ligand in standard methods. To customize an immobilization method, select ***Enter custom mode*** in the ***Method definition*** step. Custom mode adds the following features to the method definition:

- Parameters can be changed for most commands.
- Commands can be added, copied, moved and deleted in the command sequence.

Follow the instructions below to use these customization features.

Action	Instruction
Change command parameters	1. Select the command in the command sequence. 2. Change the parameter settings as required.

Action	Instruction
Add a new command	Select ***Add command*** and choose the command type. Available types are ***Injection*** and ***Wash***. The new command will be added at the end of the command sequence.
Copy a command	Click ***Copy*** on the command. A copy of the command will be added to the command sequence. You can change the command name for all commands except ***Wash*** and ***Ligand***.
Move a command	Drag the command to the new position in the command sequence.
Delete a command	Click ***Remove*** on the command.

Note: *A single ligand command is required in each immobilization step.*

Aim for target level

The option ***Aim for target level*** performs a test injection of ligand over the non-activated surface to estimate the rate of preconcentration, determined by the electrostatic attraction between ligand and surface. The surface is washed to remove traces of ligand and then activated. Pulses of ligand are injected over the activated surface, using ligand contact times based on information from the test injection. The procedure continues until the target level is reached, comparing the responses after the last ligand injection and before activation, or until the total ligand volume in the microplate is consumed. The surface is deactivated and the immobilization results presented, including information about whether or not the target level was reached.

The test injection can be removed in customized methods to, for example, conserve valuable ligand. The ligand is still injected in short pulses after activation, but in a more conservative manner, since no information about the preconcentration rate is available. Chip types that do not require activation, such as Sensor Chip SA, lack the test injection step by default.

Positioning and plate layout

This workspace shows the required volumes and microplate/rack positions for the method.

Change ***Id*** and ***Type*** for the plate and reagent rack if required in the graphical microplate representation panel. Select whether you want the workspace to display trays with the positioning overview, or a summary of the total volumes that need to be prepared for each solution.

Solution positions can be changed through drag and drop within limits. Solutions cannot be moved to positions that have insufficient capacity, or to positions that would require the sample tray to be changed within a cycle.

The volumes are recommended minimum values of the solutions. Extra volume for pipetting are not included in the volume summary.

6.3 Analysis methods

Introduction

Analysis methods are used to run experiments using the Biacore 1 series.

Predefined methods for a range of applications are provided with the system, and are recommended starting points for development of custom methods (see *Creating a new method*). For many purposes, the predefined methods are ready to run after sample information has been provided.

This section describes the principles of creating a method from start. Apply the principles as appropriate when editing predefined or existing methods.

In this section

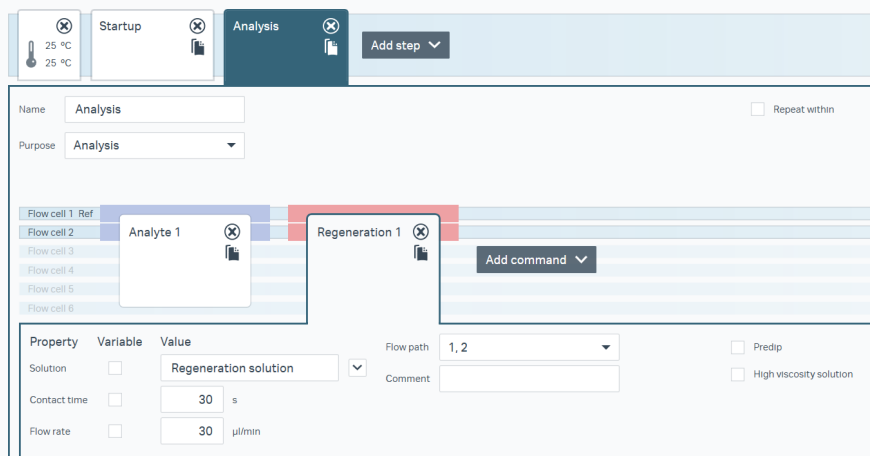
6.3.1 Method overview

Method structure

Analysis methods are built from the components summarized in the table below. Steps and commands are represented by tabs, with the currently selected tab expanded to show the tab details.

Component	Description
Steps	***Steps*** define the overall structure of the method and represent one or more repeated cycles. Steps have a specified purpose which determines how the cycle will be handled during evaluation of the results.
Commands	A ***command*** represents an individual operation, usually injection of solution over the sensor surface. The commands are sequentially ordered and determines the series of operations performed in a step.
Variables	Command parameters can be set as constant or variable. Constant parameters are applied every time the command is executed. Variables may be given different values for different cycles.

In the illustration below, the ***Analysis*** step is selected and expanded at the top, the ***Regeneration 1*** command is selected and expanded in the middle, and its command parameters are displayed at the bottom.



Method definition workflow

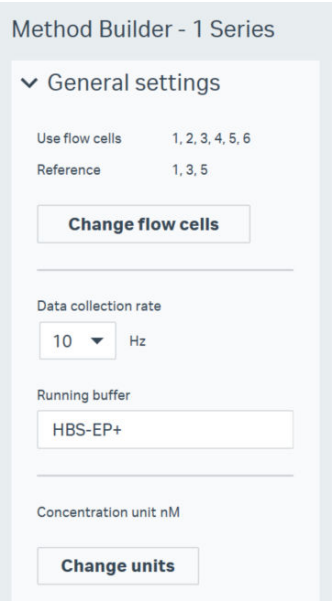
The steps below give a brief summary of the workflow for setting up analysis methods. For most purposes, you will start from a predefined method template that includes steps and commands appropriate for the method purpose. You may however want to modify the predefined steps and commands or to add new ones to the method.

Note: *The flow cell temperature in predefined method templates is generally set to 25°C. To change temperature, add or edit a* ***Set temperature*** *step. The temperature can be set multiple times within a method.*

Step	Action
1	Define the steps in the method. For each step, set up the command sequence that will determine operations in the step. ***Tip:*** *Steps can be copied to re-use the same command sequence.*
2	Assign variable parameters. This can be done either by entering variable values manually, or by importing variable information from the Windows clipboard or an external file. Corresponding cycles are automatically added. Cycles that have no variable parameters must be added manually to the respective steps.
3	Adjust the positions of solutions in the microplates if required. Positions are assigned automatically for solutions that are fixed in the method definition and for variable solutions that are entered manually. Imported sample data may include positioning information (for example, when the information is obtained from a laboratory robot used to prepare the microplates).
4	Examine the ***Cycle overview*** to ensure that samples are run in the desired order (in particular, that repeated cycles are appropriately placed). The cycle overview also displays the estimated duration of the run.
5	Save the method if required.
6	Use the ***Plate layout*** information to prepare the microplates if required, then send the run to the activity queue.

6.3.2 General settings

General method settings are specified to the left of the ***Method definition*** workspace.



Setting	Description
Flow cells	Click ***Change flow cells*** to specify which flow cells to use in the method and to select the reference. Flow cells that are not used are visualized by a lighter color in the command sequence. Maximum two flow cells and one reference are possible with Biacore 1K. More alternatives are available for Biacore 1K+ and Biacore 1S+, which also allow a serial flow through four or six flow cells and the selection of multiple references. The references will be coupled pairwise to the closest downstream flow cell if only odd reference flow cells are used (1, 3, 5). Otherwise, multiple reference subtracted sensorgrams are obtained for each active flow cell. **Example:** A Biacore 1K+ instrument is set to use flow cells 1, 2 ,3 and 4. The reference selection 1 and 3 will generate the sensorgrams 2-1 and 4-3. By instead selecting 1 and 2 as references, sensorgrams 3-1, 3-2, 4-1 and 4-2 are generated.

Setting	Description
Data collection rate	The number of data points registered per second from each flow cell during a run. Choose between 1, 10 or, for Biacore 1S+, 40 Hz. 1 Hz is sufficient for most general applications. Use 10 Hz or 40 Hz when resolution of fast binding events is important.
Running buffer	Enter a name for the running buffer if required. This name will be shown in the results and run documentation.
Concentration unit	Click ***Change units*** to select the unit(s) that will apply for concentration values entered in the method. The unit can vary between command types.

6.3.3 Method steps

Introduction

Steps define the overall structure of the method and represent one or more repeated cycles. Steps have specified purposes which determine how the step will be handled during evaluation of the results. The assigned purpose does not affect how the step will be run.

Adding a new step

Follow the instructions below to add a new step to the method.

Step	Action
1	Select ***Add step*** and choose the step purpose (see *Step purposes, on page 87*). You can change the step purpose if required after the step has been added.
2	Provide a name for the step. ***Tip:*** *New steps are named by default with the step purpose and a serial number (for example, **Analysis 2**). Changing to a more informative name can help in understanding the method structure.*
3	Select ***Repeat within*** if the step is to be repeated within the context of another step (referred to as the ***parent step***). Choose the parent step in the ***Repeat within*** field, and choose between repeating at a set frequency or distributing a set number of repeated steps evenly between the cycles in the parent step. Choose also whether to perform the step additionally at the beginning and end of the parent step. Use this option for cycles such as solvent correction or control samples that are repeated at regular intervals throughout the run. Examine the ***Cycle overview*** to see how the repeated step will be distributed through the run (see *Section 6.3.8 Cycle overview, on page 106*). ***Note:*** *If you want to run the repeated step only at the beginning and/or end of the series, check the appropriate option and set either the number of distributed occurrences to 0 or the frequency of occurrence to a number higher than the number of cycles in the series.*
4	Define the command sequence for the new step (see *Section 6.3.4 Command sequence, on page 90*).

Step purposes

One of the following purposes is assigned to each step in a method:

Purpose	Description
Conditioning	Used to condition the sensor surface at the start of an assay. Conditioning at the beginning of a method is required, for example, for some pre-immobilized sensor chips such as Sensor Chip SA.
Startup	Used to equilibrate the flow system and sensor surface at the start of an assay, before the first sample is analyzed. The first few cycles on a newly immobilized or docked chip often show drifting response as the system stabilizes, and startup steps prevent such drift from affecting the first analysis cycles. Startup cycles should preferably use the same injection conditions as analysis steps.
Calibration	Used for calibration cycles in concentration analysis applications. Calibration curves are created from samples run in ***Calibration*** steps. ***Calibration*** steps are only available if the ***Concentration & Potency*** extension is active.
Analysis	Used for sample analysis.
Solvent correction	Used specifically for solvent correction cycles. The step contains one ***Solvent correction*** command (see *Solvent correction command, on page 95*), and should typically be repeated at intervals throughout the assay.
Rmax control	Used specifically for determining the analyte binding capacity of the surface by injecting a high concentration of a known binder. This information is used in evaluation of weak binding, most commonly in work with fragments and small molecules (see the *Biacore Insight Evaluation Software Manual* for details). ***Rmax control*** steps are only available if the ***Extended screening*** extension is active.
General	Used for steps that do not fit any of the other purposes.

Managing steps

Follow the instructions in the table below to copy, move and delete steps in the method.

Action	Instruction
Copy a step	Select  ***Copy*** in the step. A copy of the step including its command sequence is added to the step sequence. Any steps repeated within the step being copied are also copied.
Move a step	Drag the step to a new position in the step sequence. A repeated step cannot be moved separately from its parent step.
Remove a step	Select  ***Remove***. If a parent step is removed, the child steps are also removed.

6.3.4 Command sequence

Introduction

The command sequence in a method step defines the operations that will be performed in each cycle in the step. Manage the command sequence in the same way as the step sequence (see *Managing steps, on page 88*).

The various injection commands differ in the way the injection is performed and in how the cycles are handled in evaluation of the results. More detailed descriptions are given in the sections that follow.

Command names

Commands are automatically named with a serial number. If there are more than one command of the same type they are always sequentially numbered, that is, ***Wash1*** is executed before ***Wash2***. An automatic renumbering is done if the order is changed.

The name of a command cannot be altered, but a comment can be added. The comment is displayed below the command name.

Fixed and variable values

Many command settings may be either fixed or variable. Values for fixed settings are entered directly in the command definition and apply every time the command is executed. Values for variable settings are entered in the ***Variables and positioning*** step, and determine the number of cycles that will be run in the step. Variables may refer to sample names or command parameters such as flow rate or contact time.

User-defined variables can be added to some commands.

As an example, the analyte injection command in a sample analysis step may have fixed values for ***Contact time*** and ***Dissociation time***, and variable values for ***Solution*** (identifying the sample that will be injected) and ***Concentration*** (used in evaluating the results). A user-defined variable may be added, for example, to hold the batch number of the sample.

General command settings

General command settings are described in the table below. Settings that are specific to one or a few commands are described in the detailed command descriptions that follow this section.

Setting	Description
Solution	Name of the solution to be injected.
Contact time	Contact time for the injection.
Dissociation time	Time after the end of the injection to allow analyte dissociation before the next command is executed.

Setting	Description
Flow rate	Flow rate, maintained for the duration of the command.
Flow path	Flow path for the injection. Which options that are listed depend on which flow cells are used in the method. The choice is illustrated graphically by colored bars in the command sequence.
Predip	Check this option to dip the needle in a separate well containing the injection solution before aspirating the solution to be injected. This will rinse the needle tip briefly to minimize carry-over effects from the previously injected solution.
Mix	Mix the sample with another solution before injecting. Specify the name of the solution to be mixed and the percentage in the final mixture (for example, specifying ***Mix with 20% buffer*** will mix 8 parts of sample with 2 parts of buffer). ***Note:*** *Mixing is performed in the reagent rack or microplate and requires an empty vial respectively plate position. The empty vial or position must be on the same tray as the solutions to be mixed.* Mixing is supported in vials with 7 or 11 mm in diameter, 96-well microplates with well volume 250 or 650 µL, and 384-well microplates with well volume 110 or 200 µL.
Concentration	(Optional) Enter the sample concentration or check this option to include concentration as a variable for the injected solution. The concentration can be written in scientific format. ***Note:*** *Do not include concentrations as part of the sample name. This will affect grouping by sample in the Evaluation Software.*
Molecular weight	(Optional) Enter the sample molecular weight or check this option to include molecular weight as a variable for the injected solution.

Setting	Description
Dilution	(Optional) Check this option to include sample dilution as a variable for the injected solution. The ***Dilution*** value is used for calculating the original concentration from concentration measurements on diluted samples in evaluation of concentration measurements. The value is taken as 1 if the field is left blank ***Dilution*** is only available for ***Analyte*** and ***General*** commands, and only when the ***Concentration & Potency*** extension is active.
Add variable	Select this option to add a user-defined variable. Provide a name for the variable and specify the format (***Text*** or ***Numeric***). Numbers entered in a text variable will be treated as text for evaluation purposes.

6.3.5 Command descriptions

Introduction

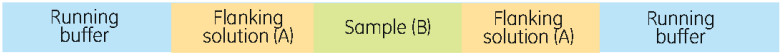
This section describes the commands that can be used in analysis methods. See *General command settings, on page 90* for settings that are common to several commands.

Commands are listed here in alphabetical order.

A-B-A command

This command injects analyte ("***B***") flanked by a different solution ("***A***") which may be buffer (other than running buffer) or another sample. Use this option for example for buffer scouting without changing running buffer or for investigating interactions in the presence of different cofactors. Contact times for flanking solution before and after the sample are set separately. The same flow rate is used throughout the injection. A pre-wash between aspiration of solutions A and B can be included, which minimizes the risk for carry-over.

Dissociation times are not supported for A-B-A injections. The ***Post-analyte contact time*** for flanking solution is equivalent to a dissociation time for the analyte.



Analyte command

This command is intended for injection of analyte.

Choose the type of injection from the following options.

Type	Description
High performance	Optimizes the injection for best performance. This option slightly increases sample consumption.
Low sample consumption	Optimizes the injection for low sample consumption. This injection type is adequate for most applications.
Fast injection	Optimizes the injection for minimum overhead time. This injection type is primarily intended for ***Clean Screen*** runs in fragment screening (see the *Biacore Insight Evaluation Software Manual* for a description of ***Clean Screen***). The ***Fast injection*** type is only available if the ***Extended screening*** extension is active.

Capture command

This command is intended for injection of ligand over a capturing molecule at the beginning of a cycle. The injected solution, contact time and flow rate can be set as variables.

The ***Capture*** command injects solution by default over the first active flow cell in the series, unless it has already been assigned in a previous ***Capture*** command.

Carry-over control command

This command injects a 20 s pulse of buffer over all used flow cells at a flow rate of 30 µL/min, in order to determine whether there is carry-over of analyte or other material from the preceding command. The command is suitably placed at the end of the cycle. The command has no user-definable settings.

Dual command

This command injects first analyte A and then analyte B, without buffer in between. The contact times for the two injections are set separately. The same flow rate and flow path is used for the entire command. A pre-wash between aspiration of solutions A and B can be included, which minimizes the risk for carry-over.

Note: *Kinetic fitting can be performed to the B-part of the **Dual** command, using the 1:1 dissociation model.*

Enhancement command

This command is intended for injection of a secondary enhancement reagent following the sample injection. Enhancement reagents are most commonly used to confirm the identity of the bound analyte.

Note: *In other techniques, such as immunoassays, enhancement may be used to amplify the sample response. Due to the high sensitivity of the system, this is seldom relevant in Biacore 1 series applications.*

General command

This command is a general-purpose injection that supports the same settings as the ***Analyte*** command (see *Analyte command, on page 93*). ***General*** injections are not automatically recognized as analyte injections for evaluation purposes, but can be selected for evaluation in the Biacore Insight Evaluation Software.

Poly command

This command injects three to five analytes in sequence, without buffer in between. The contact times for the injections are set separately, with a dissociation time for the last injected analyte. Toggle between the tabs to specify the properties of each injection. The same flow rate and flow path is used for the entire command. It is not possible to run a ***Poly*** command in a single flow cell.

Note: *It is not possible to estimate concentration, kinetics or affinity from Poly inject data in Biacore Insight Evaluation Software.*

Regeneration command

This command is intended for injection of a regeneration solution following the sample injection.

Select ***High viscosity solution*** if the regeneration solution has a relative viscosity higher than about 3 (corresponding to about 35% glycerol or 40% ethylene glycol at 20°C). This will adapt the solution aspiration and injection procedure to handle the higher viscosity solution.

Single-cycle kinetics command

This command injects a series of sample concentrations in the same cycle, intended specifically for single-cycle kinetics analysis (see *Kinetics and affinity, on page 46*). The samples are injected in direct sequence, separated only by the time required to prepare the next injection. A dissociation time is included after the last sample injection.

Solvent correction command

This command injects a series of solvent correction solutions in a single cycle, with a contact time of 20 s for each solution at a flow rate of 30 µL/min. Use this command in experiments where samples contain dimethyl sulfoxide (DMSO) to maintain analyte solubility.

One solvent correction command is automatically included in a method step with purpose ***Solvent correction***. Solvent correction commands cannot be added to the command sequence in any other way. Repeat the step at regular intervals (recommended at the beginning and end of the run and every 30 to 50 cycles depending on the demands of the experiment).

Solvent correction solutions consist of running buffer containing varying concentrations of DMSO, typically covering a range of -0.5% to +1.0% from the nominal DMSO concentration in the samples (for example, if the samples are prepared in 2% DMSO, the solvent correction solutions should cover a range of 1.5% to 3.0% DMSO). The recommended number of solvent correction solutions is 4: up to 8 solutions can be used.

Note: *Solutions for repeated instances of the command are placed in separate positions by default but may be pooled if desired.*

The principles and application of solvent correction are described in the *Biacore Insight Evaluation Software Manual*.

Wait command

This command pauses the method for the specified length of time. Data collection continues during the wait period.

Wash command

This command washes the flow system with the specified solution. The solution does not pass over the sensor surface.

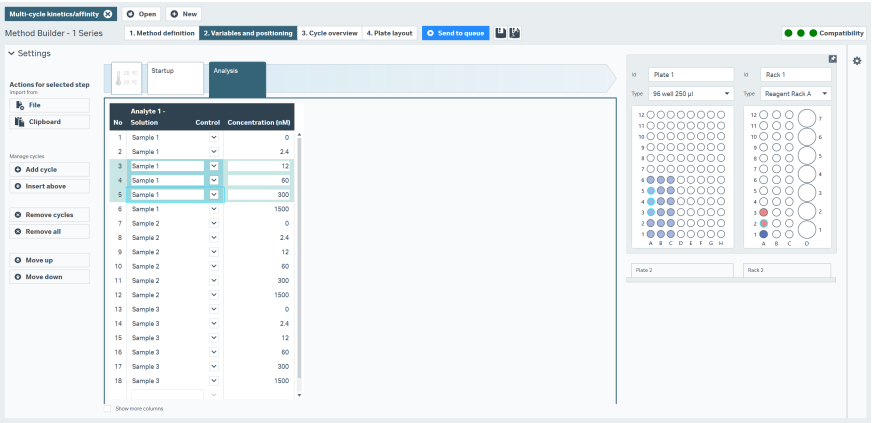
6.3.6 Entering variables and managing cycles

Introduction

Cycles are managed in the ***Variables and positioning*** step of method creation.

Cycles are added to steps in the method when variable information is provided. Variable information can be entered manually in the table or imported. New variables add as many cycles as required to hold the sample information.

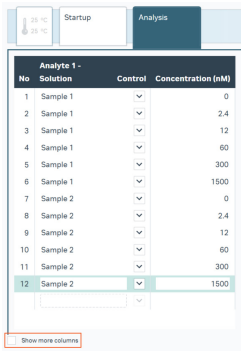
Samples and reagents are also assigned to microplate positions in this step (see *Section 6.3.7 Sample positioning, on page 102* for details).

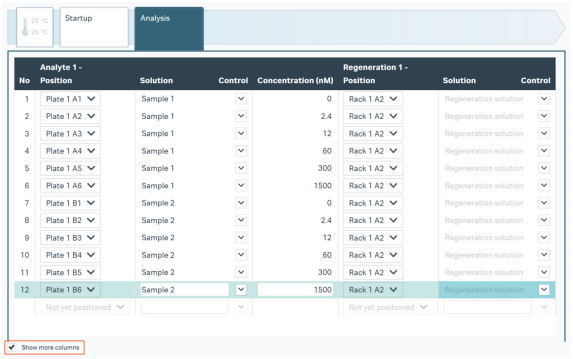


Variable table

The variable table lists variables for the commands in the currently selected step. Each row in the table represents one cycle. Click anywhere within a row to highlight the cycle.

Check ***Show more columns*** to display additional columns, including microplate positioning and information for commands in steps that do not include variables.





Default ***Show more columns***

Click ***Settings*** in the pane at the left of the variables table to collapse or expand the pane. Collapsing the pane reduces the controls to icons, to provide more area for the variable table. Some controls are hidden when the pane is collapsed.

Managing cycles

Manage cycles from the ***Settings*** pane as described in the following table. Cycles are managed independently for the different steps in a method. Click on a row in the variable table to select one cycle, or use ***Shift***-click or ***Ctrl***-click to select multiple adjacent or non-adjacent cycles respectively.

Action	Instruction
Add a cycle	Click ***Add cycle***. The cycle will be added at the end of the cycle list and corresponding microplate positions are generated automatically.
Insert a cycle	Click ***Insert above***. The new cycle will be inserted above the selected cycle(s).
Move a cycle	Cycles are executed in order from top to bottom of the list. Select one or several cycles and click ***Move up*** or ***Move down*** to move the cycle(s) up or down.
Remove a cycle	Click ***Remove cycle*** to remove the selected cycle(s).
Remove all cycles	Click ***Remove all cycles***. ***Note:*** *A method that includes a step with no cycles cannot be run.*

Entering variable values

To enter variable values manually, simply type the values in the table. Press the **Enter** key on the keyboard to add a new row in the table.

Values may be copied and pasted within or between tables using standard Windows copy-paste operations.

Importing variable values

Variable values may be imported from external text files or from the Windows clipboard. Data may be separated by tabs, commas or semi-colons. Data from Excel files may be imported via the Windows clipboard. Only one method step can be addressed in one import operation.

Columns in the source file are either mapped to variables in the target method step or set to ***Ignore***. New user-defined variables can be created by the import operation if required. If positioning information is imported, solutions will be positioned according to the specification in the source file. If samples are imported without positioning information, rows in the source file will be assigned in sequence to rows in the variable table, and positioned according to the current positioning settings in the software.

Follow the steps below to import data to the variable table. An example of variable import is shown in *Variable import example, on page 100*.

Step	Action
1	Set up the source material in a table with one sample per row and sample details in separate columns. Include microplate id and well position if you want to import samples to specific microplate positions. Use a header row to identify the columns. The content of the header row is for identification purposes only and will not be imported. The number of columns is not important provided that all detail that will be imported is represented. Additional columns can be ignored in the import operation. ***Note:*** *Do not construct the source table as a direct representation of the microplate.*
2	Select whether to import from ***file*** or ***clipboard***. For importing from a file, select the source file. For importing from the clipboard, the data must have been copied to the clipboard before the import operation is started.
3	Select the appropriate settings for the import operation:

Decimal separator	Choose ***Dot*** or ***Comma*** as appropriate.

Step **Action**

Default import to	Specify the default command to which the data will be imported. The target command can be changed individually for each imported parameter when data is mapped to variables.
Includes column headers	Check this option if the imported data includes column headers. Column headers help to identify columns but are not imported.
Remove existing cycles	Check this option if you want to clear all existing cycles from the target step. If the option is not checked, imported data will be added to the existing data as new cycles.

4 Map the columns in the imported data to variables in the method step. For each column, select the target command and the variable for import. Choose ***Ignore*** in the ***Variable*** header if you do not want to import a particular parameter. Choose ***New user defined*** to create a new user defined variable to hold imported values that are not mapped to existing variables.

If positioning information is imported, samples will be placed in the specified positions. Existing cycles that are kept will be moved to the first available positions after the imported cycles.

5 If ***Plate id*** is imported, assign plates to tray positions at the top right of the workspace.

6 In the ***Included*** column, remove the checkmark from any rows that you do not want to import.

7 Examine the parameters in the import preview carefully to ensure that the import operation is set up correctly.

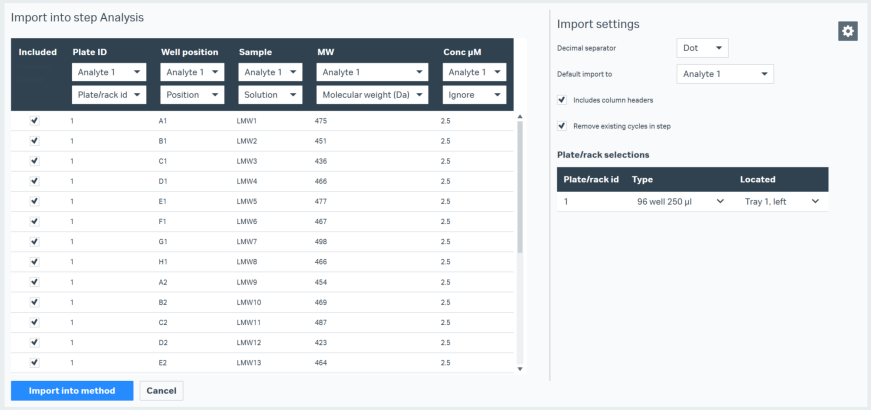
8 Select ***Import into method***.

If positioning information is imported, solutions that are imported from different rows to the same position will be pooled, provided that all other parameters are identical and that the position has sufficient capacity.

Variable import example

The example below shows part of a Microsoft Excel sheet containing sample data and a corresponding mapping into variables in a method. Note that the source information for concentration is ignored (it is not included as a variable in the method).

	A	B	C	D	E
1	Plate ID	Well position	Sample	MW	Conc µM
2	1	A1	LMW1	475	2.5
3	1	B1	LMW2	451	2.5
4	1	C1	LMW3	436	2.5
5	1	D1	LMW4	466	2.5
6	1	E1	LMW5	477	2.5
7	1	F1	LMW6	467	2.5
8	1	G1	LMW7	498	2.5
9	1	H1	LMW8	466	2.5
10	1	A2	LMW9	454	2.5
11	1	B2	LMW10	469	2.5
12	1	C2	LMW11	487	2.5
13	1	D2	LMW12	423	2.5
14	1	E2	LMW13	464	2.5
15	1	F2	LMW14	412	2.5
16	1	G2	LMW15	497	2.5
17	1	H2	LMW16	501	2.5
18	1	A3	LMW17	446	2.5
19	1	B3	LMW18	481	2.5
20	1	C3	LMW19	432	2.5
21	1	D3	LMW20	469	2.5
22	1	E3	LMW21	416	2.5
23	1	F3	LMW22	466	2.5
24	1	G3	LMW23	489	2.5
25	1	H3	LMW24	427	2.5

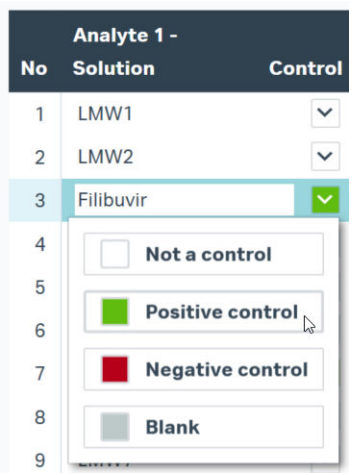


Note: *Importing values creates the number of cycles required to hold the imported values. You do not need to create empty cycles before importing values.*

Note: *Control sample status cannot be imported, but will be assigned if imported sample names have already been defined as controls.*

Setting control sample properties

Control sample properties are defined for variable solutions in the variable table, and can be set for the injected solution in most commands. The control properties of fixed solutions are set next to their sample name in the command settings in ***Method*** definition.



The control sample property affects how the sample is handled in evaluation.

Setting a property for a given sample in the variable table for one command will set the property for all occurrences of the identical solution name in all steps and commands in the method. If you want to use the same substance as a control and a sample in different contexts of the method, distinguish the usages by using different sample names.

Control sample properties can also be set in the ***Variables*** tool in the Evaluation Software (see the *Biacore Insight Evaluation Software Manual* for details).

6.3.7 Sample positioning

Introduction

Positions are managed in the ***Variables and positioning*** step. Equivalent functions are available for immobilization methods in the ***Positioning and plate layout*** step.

Samples and other solutions are automatically assigned to positions in microplates or racks in accordance with the ***Positioning settings*** in the method. A general rule is that all solutions that will be used within a cycle need to be positioned on the same tray. Occupied positions are colored according to their positioning group in ***Positioning settings***.

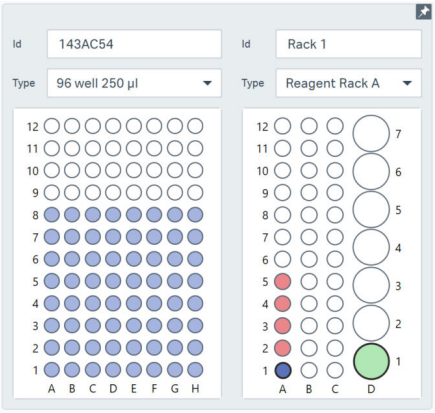
If you need to have a specific positioning layout, sample information including positions can be imported from an external source. Imported positions are locked and protected from automatic rearrangement by the software.

For re-arrangement of non-imported positions, use the functions available in ***Positioning settings***. For minor re-arrangements, solutions can be moved manually using drag and drop.

Positioning information can be printed from the ***Plate layout*** step as a guide to microplate preparation.

Position identification

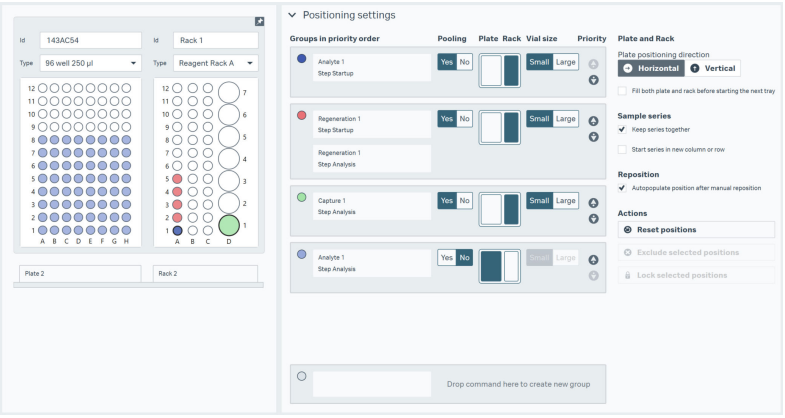
Microplate positions are identified by plate id and well coordinates (e.g., ***Plate 1 G3***). Microplates are numbered sequentially by default. Choose the microplate ***Type*** and change the ***Id*** if desired in the plate representation on the right of the workspace. Set ***Type*** to ***None*** to disable the plate or rack.



Note: *You may use a barcode reader to enter the microplate **Id** if you are using coded microplates. The microplate **Type** is not however linked to the microplate barcode. Make sure that the correct **Type** is selected.*

Positioning settings

Click ***Positioning settings*** to access and change settings that determine the automatic positioning of samples. Positions that have been assigned automatically are rearranged directly if the settings are changed, unless the function is switched off (see below).



Solutions may be grouped together so that the settings apply to all solutions in the group. To split a group, drag one of the commands from a group to the empty placeholder at the bottom of the group list. To combine solutions in a group, drag the solutions together. The following settings apply separately to groups:

Setting	Description
Pooling	Select ***Yes*** to allow multiple injections to be taken from the same microplate well. Pooling requires that the samples have the same variables, such as the solution name and concentration unit. ***Note:*** *Pooling is only relevant for solutions that are used repeatedly.*
	Select whether the samples should be taken from the plate or rack.
Vial size	Choose if samples in the rack should be positioned in the smaller or larger vial type. ***Note:*** *The volume of the small vials differ depending on rack type.*

Setting	Description
	Use these icons to change the priority of positioning groups in the group list. Solutions are positioned in the microplate in order, from top to bottom of the list. Changing the order in the list will affect the positioning. If available positions are few, a group high up on the list will be prioritized and get its desired positioning, whereas a group far down may have to compromise.

The following settings apply to all solutions, unless specified otherwise:

Setting	Description
Plate positioning direction	Select whether the plate should be filled vertically – column by column – or horizontally – row by row.
Fill both plate and rack before...	Check this option to position samples in both the microplate and rack wherever there are free positions before samples are added to the second tray. This option is only available for Biacore 1K+ and Biacore 1S+.
Keep series together	Check this option to position samples within a series in the same microplate as far as possible. A series is a group of samples with the same name that differ in another variable, such as concentration or dilution.
Start series in new column or row	Check this option to start each sample series in a new plate column (***Vertical***) or row (***Horizontal***). For the definition of series, see above.
Autopopulate position after manual reposition	Choose this function to automatically rearrange positions where possible when manual changes are made. Automatic rearrangement may be necessary if solutions are manually repositioned to a different tray or to already occupied wells.
 ***Reset positions***	Choose this function after manually repositioning solutions to restore all solutions in the microplates to the positions determined by the current positioning settings. Solutions imported with positioning information are not affected.
 ***Exclude selected positions***	This option makes selected positions inaccessible for both automatic and manual positioning. Exclusion of positions can be suitable when partly used microplates are reused, to ensure that nothing is positioned in previously used wells. For selection of positions, see *Selecting positions, on page 105*.

Setting	Description
Lock/ unlock selected positions	Choose this function to lock positions and protect them from automatic rearrangement. Locked positions are marked with a bold border. Select the locked positions and click on the same button again to unlock them.

Selecting positions

Positions can be selected from the graphical tray representation using the actions described below, or by selecting their corresponding cycles in the variable table (see *Managing cycles, on page 97*).

Action	Instruction
Select a single position	Click on a position.
Select a group of adjacent positions	Drag around an area to select all positions within the area.
Select a group of non-adjacent positions	***Ctrl***-click on multiple positions to select them one by one. Drag around multiple areas while holding ***Ctrl*** to select all positions within the areas.

Moving solutions manually

To change position, drag the selected solution(s) to a new position in the graphical microplate and reagent rack illustration.

Alternatively, follow the steps below:

Step	Action
1	Select ***Show more columns*** to expand the sample table and show the ***Position*** column.
2	Click on the drop-drown menu for the position you want to move. Specify tray.
3	Choose the position in the blank microplate illustration that appears.

Solutions cannot be moved in such a way that would require sample tray change within a cycle (for example, an analyte solution and a regeneration solution that are used in the same cycle cannot be on different trays).

Existing positions are rearranged according to ***Positioning settings*** when manual changes are made, unless ***Autopopulate position...*** is inactive. Positions that have been imported or manually moved are marked with a heavy black border in the microplate and rack illustration and are protected from auto-rearrangement.

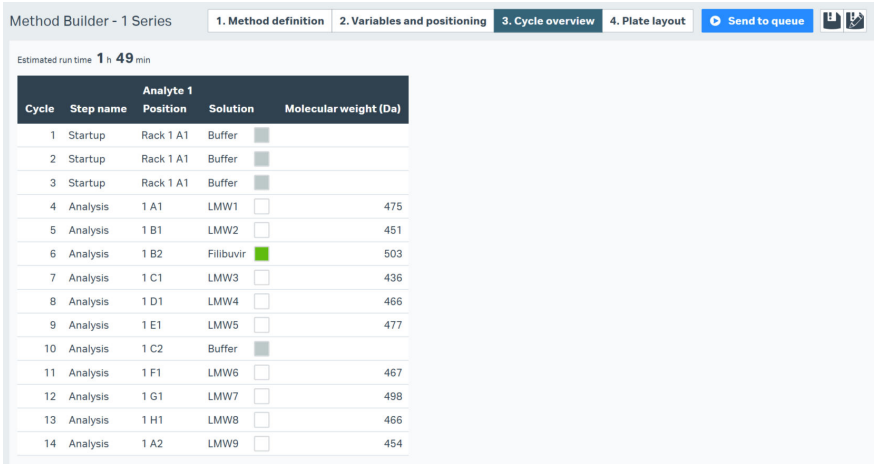
6.3.8 Cycle overview

Introduction

The ***Cycle overview*** step provides an overview of the cycle order and the estimated run time for the run. Use this information to check that the run is set up as intended, in particular for steps that are repeated in the context of other steps.

Note: *The estimated time for the run shown in* ***Cycle overview*** *does not include preparation steps such as temperature change, and may differ from the time shown when the method is added to the activity queue.*

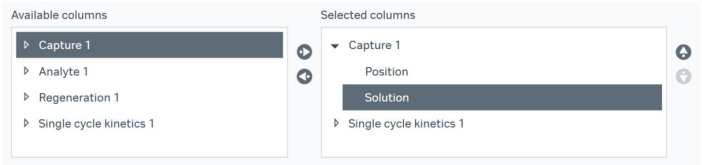
You cannot make any changes to the cycle order or parameter values in this step.



Cycle	Step name	Analyte 1 Position	Solution	Molecular weight (Da)
1	Startup	Rack 1 A1	Buffer	
2	Startup	Rack 1 A1	Buffer	
3	Startup	Rack 1 A1	Buffer	
4	Analysis	1 A1	LMW1	475
5	Analysis	1 B1	LMW2	451
6	Analysis	1 B2	Filibuvir	503
7	Analysis	1 C1	LMW3	436
8	Analysis	1 D1	LMW4	466
9	Analysis	1 E1	LMW5	477
10	Analysis	1 C2	Buffer	
11	Analysis	1 F1	LMW6	467
12	Analysis	1 G1	LMW7	498
13	Analysis	1 H1	LMW8	466
14	Analysis	1 A2	LMW9	454

Settings

Click ***Expand/Collapse*** at the right of the workspace to select which columns are displayed and in what order.



- Columns are grouped by command. Use the symbol at the left of the command name to expand or collapse the list of individual columns.
- Select a command or column and use the arrows to move the selection between ***Available columns*** and ***Selected columns*** and/or to reposition the selection in the display.

6.3.9 Plate layout

Description

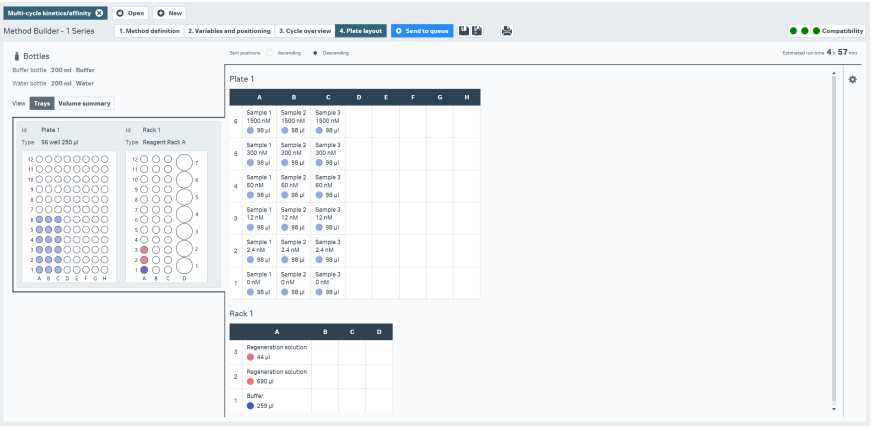


Plate layout provides a summary of the sample positioning in microplates and required solution volumes, as an aid when preparing samples. Select whether you want the workspace to display trays with microplates and racks (select ***Trays***), or a summary of the total volumes that need to be prepared for each solution (select ***Volume summary***).

For trays, you can sort the table rows in ascending or descending order of position. Click ***Expand/Collapse*** to select what variables to include in the tables. The plate layout display cannot be edited in any other respect. Make any required adjustments to the plate layout in the ***Variables and positioning*** step (see *Section 6.3.6 Entering variables and managing cycles, on page 96*).

Note: *The graphical representation of the microplates is not affected by the table sort order.*

Volumes listed in the plate layout are recommended minimum values. Extra volumes for pipetting are not included in the volume summary.

Click ***Print*** to print a copy of the current workspace.

7 Runs workspace

About this chapter

This chapter describes the ***Runs*** workspace, where the results of ongoing and stored runs are displayed.

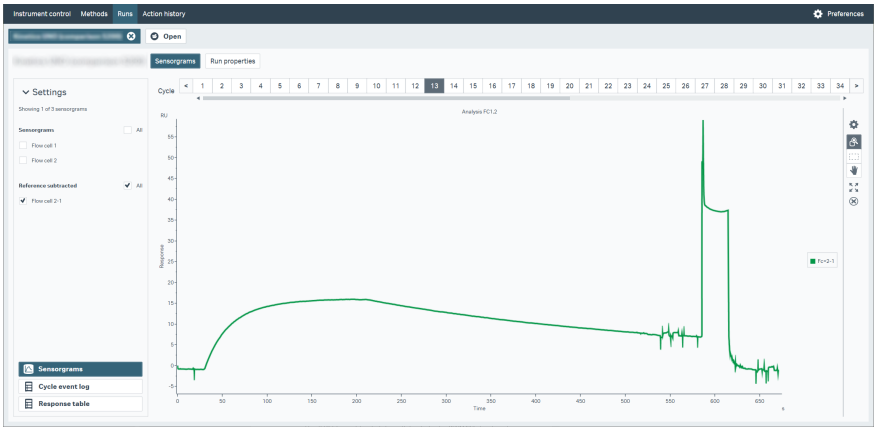
In this chapter

7.1 Opening a run

Procedure

Ongoing runs are displayed automatically in the ***Runs*** workspace (see *Section 5.5 Display during a method run, on page 68*).

Finished runs are opened from the database. Follow the procedure below to open a finished run.



Step	Action
1	Select ***Open*** if the list of runs is not displayed.
2	Navigate to the required folder or use the ***Search*** function (see *Searching for database objects, on page 16*).
3	Double-click on the required run or choose the run and select ***Open*** at the bottom right of the workspace.

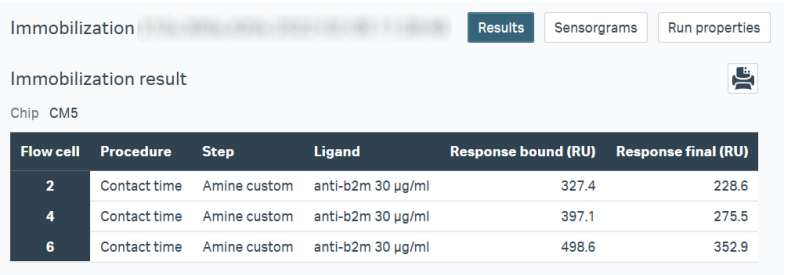
7.2 Display information

The ***Runs*** workspace displays two or three kinds of information for the selected run. Use the buttons at the top of the workspace to choose the information to display.

In this section

7.2.1 Results display

The ***Results*** display is only shown for immobilization runs, and certain tools such as ***System check*** that generate numerical and/or text results.



Flow cell	Procedure	Step	Ligand	Response bound (RU)	Response final (RU)
2	Contact time	Amine custom	anti-b2m 30 µg/ml	327.4	228.6
4	Contact time	Amine custom	anti-b2m 30 µg/ml	397.1	275.5
6	Contact time	Amine custom	anti-b2m 30 µg/ml	498.6	352.9

For immobilization runs, two response levels are reported. ***Response Bound*** is the difference in response level directly before and after the ligand injection. ***Response Final*** is the difference in response level before the activation injection and after the deactivation injection, and is used as the immobilization level in ***Chip information*** details.

7.2.2 Sensorgram display

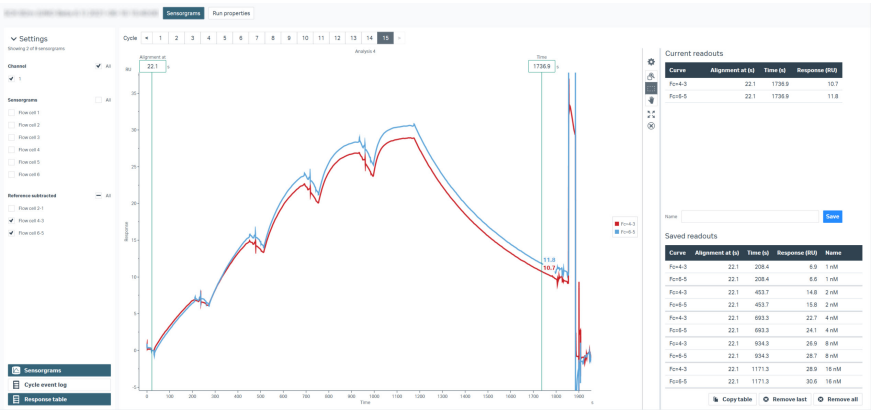
The sensorgram display shows the sensorgram details for one cycle at a time. Choose the cycle to display from the bar at the top of the panel.

Tip: *Select a cycle in the cycle bar and then use the left and right arrow keys on the keyboard to browse rapidly through the cycles.*

The sensorgram display may contain up to four subpanels, for ***Sensorgrams***, ***Cycle event log***, ***Report point table***, and ***Response table***. Use the buttons at the bottom of the ***Settings*** panel to control which subpanels are displayed.

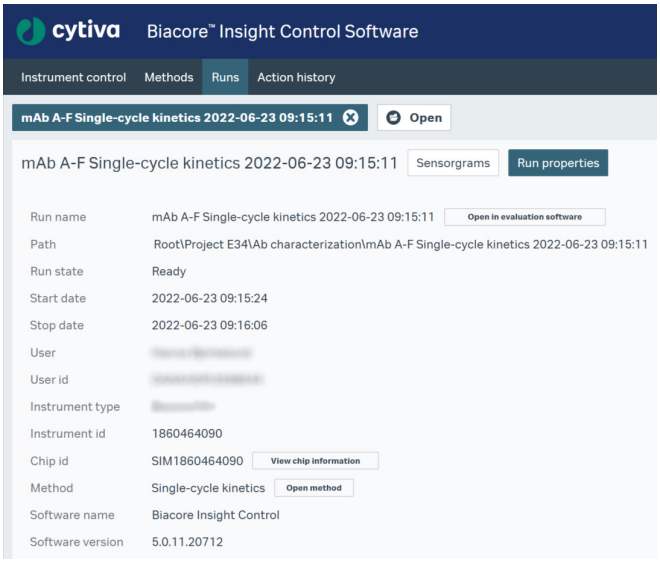
Subpanel	Description
Command sequence	Displays the commands of interactive runs. Not visible in method runs.
Sensorgrams	Displays the sensorgram curves as selected in the Settings panel at the left of the workspace.
Cycle event log	Displays a detailed list of instrument control events in the current cycle. Events are marked and identified on the sensorgram display.
Report point table	Displays the report point table for immobilization runs. This button is not available for analysis runs. Report points for analysis runs are created in the Evaluation Software.
Injection pause controls	Enables pausing of the ligand injection during immobilization, or any injection during interactive runs. Not visible for analysis runs. Disabled in ***Runs*** workspace.
Response table	Displays a list of response levels for selected curves at the position of the ***Response ruler***. Response levels are relative the alignment point, as indicated by the ***Alignment ruler***, when alignment is enabled. See *Chart display settings, on page 115* for details about the rulers. It is possible to save the response level readout together with a name, which are stored in the result file. ***Note:*** *The response level readout table cannot be changed in finished regulated GxP runs.*

Use the view settings to control the content of the ***Sensorgrams*** display. See *Section 7.3 Sensorgram view settings, on page 115* for details.


Sensorgrams
Run properties
Settings
Cycle
Current readouts
Curve
Alignment at (s)
Time (s)
Response (RU)
Fc4-3
Fc6-5
22.1
1736.9
10.7
11.8
Name
Save
Saved readouts
Name
1 nM
2 nM
4 nM
8 nM
16 nM
Sensorgrams
Cycle event log
Response table
Copy table
Remove last
Remove all

7.2.3 Run properties display

Run properties shows details of the run.



The following functions are available from ***Run properties***:

Function	Description
Open in Evaluation Software	Opens the run in a new instance of the Evaluation Software. If this function is used for an ongoing run, data up to and including the most recently completed analysis cycle is opened in the Evaluation Software. Evaluation of data from an ongoing run cannot be saved or exported from the Evaluation Software.
View chip info	Displays sensor chip information in a separate window.
Open method	Opens the method for the run in the ***Methods*** workspace. ***Note:*** *This function opens the copy of the method stored in the result file, that was actually used when the run was executed. Any later changes that might have been made to the method in the database are not included.*

7.3 Sensorgram view settings

Introduction

The content and appearance of the sensorgram display is controlled in three ways:

- Use the ***Settings*** panel at the left of the workspace to control which sensorgrams are displayed.
- Use the zoom and selection tools at the right of the workspace to shift between zoom and sensorgram selection options.
- Use ***Chart settings*** at the right of the workspace to control how the run data is displayed.

In addition, you can drag over an area of the sensorgram display to zoom in to that area.

Settings

Select the channels and sensorgrams you want to include in the display. All options are selected by default.

Zoom and selection tools

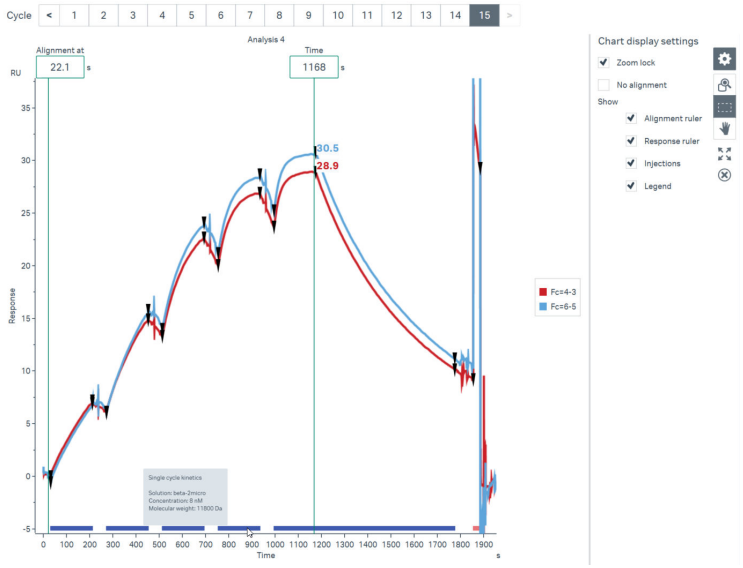
The following options for zooming and selection are found to the right of the workspace:

Setting	Description
Zoom mode	Enables display zooming. Drag with the mouse to enlarge a selected portion of the sensorgram display. Double-click in the display to restore the previous zoom level.
Select area mode	Enables selection by dragging over an area with the mouse. All curves 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 sensorgrams in the panel.

Chart display settings

Click ***Chart settings*** at the right of the workspace to access the following options:

Option	Description
Zoom lock	Maintains the current zoom factor when cycle or sensorgram selection is changed. If this option is not checked, the display will zoom automatically according to the current display data when selections are changed.
No alignment	Disables sensorgram alignment.
Show: Alignment ruler	Displays a cursor indicating the time point at which all curves are aligned to zero response when alignment is enabled. The ruler position does not change between cycles.
Show: Response ruler	Displays a cursor indicating the time point from which the response levels of selected curves are extracted, as presented in the sensorgram display and in the ***Response table***. The ruler position does not change between cycles.
Show: Injections	Highlights the duration of injections with a heavy colored line and adds injection key point markers. Information about the injection is displayed when hovering over the line.
Show: Legend	Displays a legend with sensorgram identification.



8 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.*

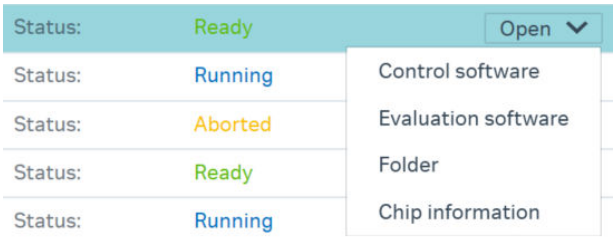
Action	Instruction
Change number of visible actions	Set a number in the ***Show ... items*** drop-down list. Use the page selection buttons to step through the log.
Sort on date	Click on the heading of the date column to toggle between oldest or newest first.
See new actions	Click ***Refresh***. New 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.



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