Regeneration Scouting Kit

Instructions for Use

Product description

Product code:	BR100556
Contents:	• Ethylene glycol (p.a.), 11 mL • Glycine-HCl, 10 mM, pH 1.5, 11 mL • Glycine-HCl, 10 mM, pH 2.0, 11 mL • Glycine-HCl, 10 mM, pH 2.5, 11 mL • Glycine-HCl, 10 mM, pH 3.0, 11 mL • Magnesium chloride ($MgCl_2$), 4.0 M, 11 mL • Sodium hydroxide (NaOH), 0.2 M, 11 mL • Sodium dodecyl sulphate (SDS), 0.5%, 11 mL • Sodium chloride (NaCl), 5.0 M, 11 mL • Surfactant P20, 20 mL
Storage:	2°C to 8°C, except for SDS and ethylene glycol, which should be stored at room temperature. ***Note:*** *SDS and ethylene glycol precipitate at low temperature.*
Safety:	For use and handling of the product in a safe way, please refer to the Safety Data Sheet.

Intended use

Regeneration Scouting Kit, which contains common regeneration solutions, is suitable for scouting of optimal conditions for regeneration of ligands or capturing molecules attached to the sensor chip. The volumes of the solutions in this kit are generally sufficient for scouting and verification of regeneration conditions for one interaction pair.

Regeneration scouting using this kit is supported in predefined run and evaluation methods in all Biacore™ systems.

Introduction

Regeneration is the process of removing analyte, or for capture assays, ligand-analyte complexes, bound to the sensor chip without affecting the activity of the attached ligand or capturing molecule. Finding optimal regeneration conditions is a crucial part of assay development. Regeneration Scouting Kit offers convenient regeneration scouting as it contains solutions covering a wide range of conditions suitable for regeneration of varying types of ligands or capturing molecules (see table below).

Condition	Solution
Low pH	Glycine-HCl
High pH	NaOH
Ionic strength	NaCl $MgCl_2$
Detergent	SDS (ionic) Surfactant P20 (non-ionic)
Other	Ethylene glycol

See below for general guidelines on how to establish and verify regeneration conditions.

Choice of regeneration conditions

The conditions for regeneration are determined by the nature of the interaction that is to be regenerated and by the micro-environment on the surface of the sensor chip. The table below shows some suggested regeneration solutions for different ligands, in recommended order.

Note: *Avoid using Magnesium chloride ($MgCl_2$) as regeneration solution in combination with phosphate running buffers such as PBS in order to avoid precipitation of Magnesium phosphate. For Biacore 8K and Biacore 8K+, the combination of $MgCl_2$ and phosphate running buffers results in a short life length of the syringe pump.*

Ligand	Condition	Solution
Protein ligand	Low pH	10 mM glycine-HCl, pH 1.5 to 3.0
	Ethylene glycol	50%, 75%, or 100%
	High pH	1 to 100mM NaOH
	High ionic strength	1 to 4 M $MgCl_2$
		0.5 to 5 M NaCl
	Ionic detergent	0.02% to 0.5% SDS
Low molecular weight ligand	High pH with acetonitrile	20 to 100 mM NaOH + 30% acetonitrile [1]
	High pH with detergent	20 to 100 + 0.5% Surfactant P0 or 0.05% SDS
	Low pH	10 mM glycine-HCl, pH 1.5 to 3.0
	High ionic strength	1 to 4 M $MgCl_2$
Nucleic acid ligand with protein analytes	High ionic strength	1 to 5 M NaCl
	Ionic detergent	0.02% to 0.5% SDS
	Low pH	10 mM glycine-HCl, pH 1.5 to 3.0
Nucleic acids ligand with nucleic acid analytes	High pH with high ionic strength	50 mM NaOH + 1 M NaCl

[1] Acetonitrile is not included in Regeneration Scouting Kit. Always use p.a. grade reagents. Solutions of NaOH containing acetonitrile should be used within one day of preparation.

Preparation of glycine buffers

In some cases, conditions might need to be further refined for optimal performance, especially for regeneration at low pH where careful optimization can require small pH intervals.

The tables below show mixing volumes to prepare glycine buffers with pH values in 0.1 unit intervals. To prepare these buffers, larger volumes than supplied in the kit are needed. Glycine buffers in 100 mL packages can be ordered from Cytiva, see *Related products, on page 10*.

Target pH	Glycine pH 1.5 (mL)	Glycine pH 2.0 (mL)
1.6	7.0	3.0
1.7	4.6	5.4
1.8	2.7	7.3
1.9	1.2	8.8

Target pH	Glycine pH 2.0 (mL)	Glycine pH 2.5 (mL)
2.1	7.0	3.0
2.2	4.6	5.4
2.3	2.7	7.3
2.4	1.2	8.8

Target pH	Glycine pH 2.5 (mL)	Glycine pH 3.0 (mL)
2.6	7.0	3.0
2.7	4.6	5.4
2.8	2.7	7.3
2.9	1.2	8.8

Perform regeneration scouting

Follow the steps below to perform a regeneration scouting.

Step	Action
1	Make a first choice of regeneration conditions from the recommendations above. Work only with one set of conditions at a time.

Step	Action
2	Prepare the sensor chip surface with the same ligand level that is intended for use during analyses.
3	Start with the mildest regeneration solution and work towards harsher regeneration solution.
4	Use a high analyte concentration to be able to assess the effect of each regeneration solution.
5	Evaluate each regeneration solution by five repeated cycles of analyte injection, followed by 30 to 60 s injection of regeneration solution after each analyte injection.
6	Use flow rate and temperature settings that is intended for use during analyses.
7	Assess the results as described below.
8	If adequate regeneration is not achieved, evaluate another set of conditions. Alternatively, fine-tune pH or concentration of regeneration solution to improve regeneration procedures. ***Note:*** *Always use a freshly prepared sensor chip surface for scouting or optimization using a new set of conditions.*

Evaluate regeneration conditions

When evaluating regeneration conditions, it is important to assess the analyte and baseline responses.

Optimal regeneration conditions are indicated by the following observations:

- The analyte response should be within 10% of the level reached in the first scouting cycle and remains constant throughout all cycles.
- The baseline response should be constant but minor variations can be tolerated as long as the analyte response remains constant.

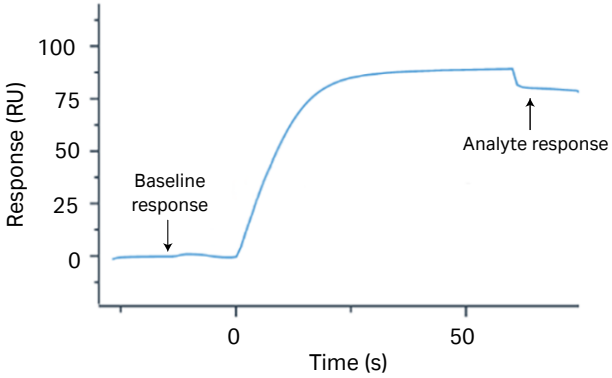
Regeneration conditions that are not optimal can cause incomplete regeneration, which are indicated by the following observations:

- Decreasing analyte response correlated with increasing baseline indicates that the analyte is not removed, and that the regeneration conditions are too mild.
- Decreasing analyte response correlated with stable or decreasing baseline indicates that ligand is being destroyed, and that the regeneration conditions are too harsh.

- Increasing analyte response can indicate that analyte remaining from previous cycles is being removed progressively, and that the regeneration conditions are slightly too mild.

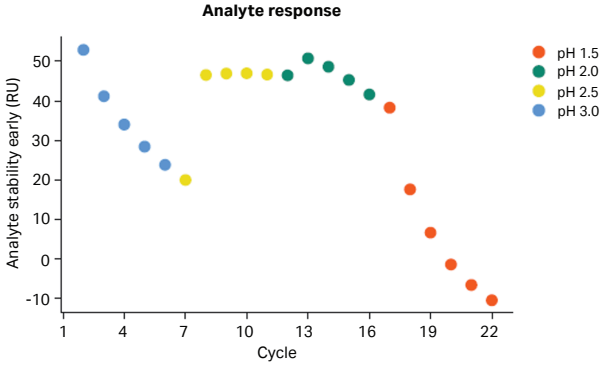
Follow the steps below to assess the conditions evaluated in the regeneration scouting.

Step	Action
1	The baseline and analyte responses are evaluated using a report point. Set report points to record the absolute baseline response just before the start of the analyte injection. Set report points to record analyte response after the end of the analyte injection (***Stability early*** or ***Stability*** depending on the instrument). See figure below.

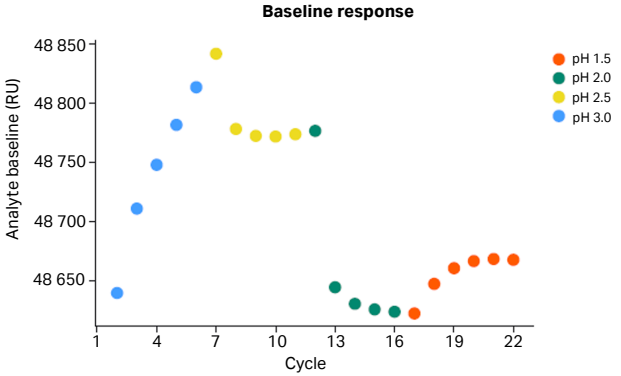


2	Prepare trend plots of baseline and analyte response levels against the cycle number, grouped by the regeneration condition. The responses in cycle 2 show the effect of regeneration performed in cycle 1, and so on. The response in cycle 1 gives a starting level for the analyte binding. See figures below for examples of trend plots. The plot below shows the analyte response against the cycle number. In this example acceptable regeneration is achieved with pH 2.5. The analyte response is stable and fairly close to the response at the start of the experiment. Higher pH is insufficient and lower pH is too harsh.

Step	Action



The plot below shows the baseline response against the cycle number. A stable baseline is obtained at pH 2.5.



Result:
pH 2.5 is identified as the most suitable regeneration condition and should be selected for further verification.

Verification

Once suitable regeneration conditions have been found, the conditions should be verified for longer series of repeated analyte binding and regeneration. Follow the steps below to verify the regeneration conditions.

Step	Action
1	Start with a freshly prepared sensor surface.
2	Run repeated identical cycles of analyte binding and regeneration. A recommended number of cycles is 20.
3	Prepare a trend plot of the analyte response against cycle number as described for scouting. Assess the results according to the regeneration requirements of the assay. For example, acceptance criteria might be set as a response in cycle 20 that is at least 90% of that in cycle 2. ***Note:*** *Some ligands show an initial decrease in response after the first cycle, so comparison of cycle 20 with cycle 1 can be misleading.*

Hints and tips

Issue	Actions
Limited amount of analyte	Perform initial scouting over a wide range of conditions with fewer steps and then focus on a narrower interval in a second scouting experiment. This can reduce the total number of cycles in the scouting process. ***Note:*** *Using fewer than three cycles for each condition can make trends in the scouting results more difficult to identify.* Reduced analyte concentration and/or flow rate can also be used to conserve analyte.

Issue	Actions
Baseline drift	Some regeneration conditions (notably SDS) can cause baseline drift immediately after the regeneration injection. Include a stabilization period after the regeneration if this behavior is observed. If SDS is used in regeneration scouting or in the final regeneration conditions, make sure the system is properly cleaned (using the ***Desorb*** routine) before running a new interaction analysis.
Precipitation at the interface	Make sure that the regeneration solutions are compatible with running buffer. For example, avoid $MgCl_2$ with phosphate-based running buffers.
Additional optimization	For most interactions, a short pulse of regeneration solution (30 to 60 s) is sufficient to strip bound material from the sensor surface. However, some interactions can require longer contact times, and testing longer regeneration contact times can be beneficial. There are also examples of double pulses of the same regeneration solution being more effective than a single long pulse. More complex samples or interactions might also be more efficiently regenerated by two consecutive pulses of regeneration solution with varying characteristics, such as low pH followed by high pH or $MgCl_2$.

Related products

10 mM Glycine pH 1.5-3.0, 50 mM NaOH, and Surfactant P20 can be ordered separately from Cytiva. See the table below for ordering information.

Product name	Content	Product code
Glycine 1.5	100 mL	BR100354
Glycine 2.0	100 mL	BR100355
Glycine 2.5	100 mL	BR100356
Glycine 3.0	100 mL	BR100357
NaOH	100 mL	BR100358
Surfactant P20	100 mL	BR100054

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For local office contact information, visit cytiva.com/contact

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