Regeneration Scouting Kit

Product description

Order code:	BR-1005-56
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 SDS and ethylene glycol should be stored at room temperature.
Safety:	For use and handling of the product in a safe way, please refer to the Safety Data Sheet.

Note: *For in vitro use only.*

Intended use

Regeneration Scouting Kit, which contains a number of common regeneration solutions, is suitable for finding optimal conditions for regeneration of the ligand attached to the sensor chip surface. The volumes of the solutions in this kit are generally sufficient for scouting and verification of regeneration conditions for one interaction pair, as described in these Instructions For Use.

Introduction

Regeneration is the process of removing bound analyte from the sensor chip after sample injection, without affecting the activity of the immobilized ligand. Finding optimal regeneration of the ligand attached to the sensor chip surface is a crucial part of assay development. Regeneration Scouting Kit offers convenient regeneration scouting as it contains a number of solutions covering a range of conditions suitable for regeneration of different ligands (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.

Preparation of glycine buffers

In some cases, conditions may need to be refined further for optimal performance. This is particularly true for regeneration at low pH where this kit supports scouting at intervals of 0.25 pH units, while careful optimization may require intervals of 0.1 pH units.

The table below gives mixing volumes for glycine buffers to obtain pH values intermediate between those supplied in Regeneration Scouting Kit.

Target pH	Glycine pH 1.5 (µL)	Glycine pH 2.0 (µL)	Glycine pH 2.5 (µL)	Glycine pH 3.0 (µL)
1.75	360	640		
2.25		360	640	
2.75			360	640

If larger volumes are needed (e.g., for preparation of buffers at intervals of 0.1 pH unit), 100 mL packages of glycine buffers can be ordered from GE, see *Ordering information, on page 9*.

Choice of regeneration conditions

Conditions for regeneration are determined by the nature of the ligand-analyte interaction and by the micro-environment on the surface of the sensor chip. Below are suggested regeneration solutions, in recommended order.

Protein ligands:

- Low pH (10 mM glycine-HCl, pH 1.5 to 3.0)
- Ethylene glycol (50%, 75%, or 100%)
- High pH (1 to 100 mM NaOH)
- High ionic strength (1 to 4 M $MgCl_2$ or 0.5 to 5 M NaCl)
- Ionic detergent (0.02% to 0.5% SDS)

Low molecular weight ligands:

- High pH with acetonitrile [1], non-ionic or ionic detergent (20 to 100 mM NaOH containing 30% acetonitrile, 0.5% Surfactant P20, or 0.05% SDS)
- Low pH (10 mM glycine-HCl pH 1.5 to 3.0)
- Magnesium chloride (1 to 4 M $MgCl_2$)

Nucleic acid ligands:

- With protein analytes:
  - High ionic strength (1 to 5 M NaCl)
  - Ionic detergent (0.02% to 0.5% SDS)
- With nucleic acid analytes:
  - 50 mM NaOH containing 1 M NaCl

Regeneration scouting instructions

Follow the instructions 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.
2	Prepare the sensor surface with the same ligand level that is intended for use during analyses.
3	Prepare 1 mL of each regeneration solution.
4	Start with the mildest regeneration solution and work towards harsher.
5	Use a high analyte concentration to maximize the efficiency of the regeneration scouting.

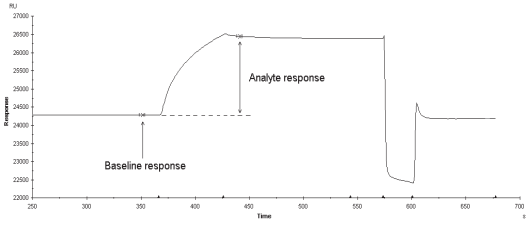
[1] Acetonitrile is not included in Regeneration Scouting Kit. Always use p.a. grade reagents. Solutions of NaOH containing acetonitrile should be used within one day of preparation.

Step	Action
6	Test each regeneration solution by repeated cycles (recommended number is 3 to 5 cycles) of analyte injection, followed by 30 to 60 s injection of regeneration solution.
7	Use flow rate and temperature settings that is intended for use during analyses.
8	Assess the results as described below.
9	If adequate regeneration is not achieved, choose a different set of conditions and repeat the scouting. ***Note:*** *Always use a freshly prepared sensor surface for scouting or optimization with a new set of conditions.*

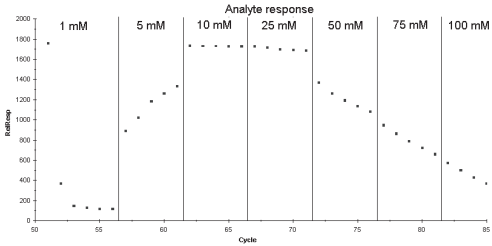
Evaluation

Follow the instructions below to evaluate the conditions tested in the regeneration scouting.

Step	Action
1	Set report points to record the absolute baseline response just before the start of the analyte injection and the analyte response relative to the baseline just after the end of the injection. See figure below for placing of report points for baseline and analyte response in regeneration scouting.

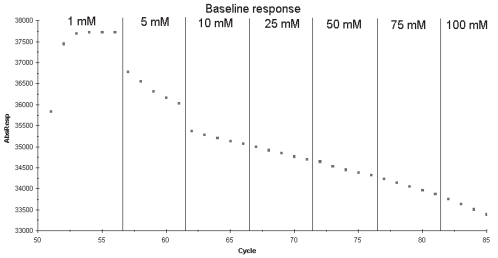


Step	Action
2	Prepare trend plots of the baseline and analyte response levels against cycle number, grouped according to regeneration conditions. The responses in cycle 2 show the effect of regeneration performed in cycle 1, and so on, and so forth. The response in cycle 1 gives a starting level for the analyte binding. See figures below for examples of trend plots. The trend plot below is showing analyte responses against the cycle number, grouped according to concentration of NaOH. Acceptable regeneration is achieved with 10 to 25 mM NaOH.



The trend plot below is showing the absolute baseline responses against the cycle number, grouped according to regeneration conditions.

Step	Action



Ideal regeneration conditions maintain a constant analyte response throughout all 5 cycles. Ideally the response should be within 10% of the level reached in the first scouting cycle and the baseline response should be constant. Variations in baseline response may be tolerated as long as the analyte response remains constant.

- Decreasing analyte response correlated with increasing baseline indicates that analyte is not removed: the regeneration conditions are too mild.
- Decreasing analyte response correlated with stable or decreasing baseline indicates that ligand is being destroyed: the regeneration conditions are too harsh.
- Increasing analyte response may indicate that analyte remaining from previous cycles is being removed progressively: the regeneration conditions are slightly too mild.

Verification

Once regeneration conditions have been found with scouting, the conditions should be verified for longer series of repeated analyte binding and regeneration.

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. Use a separate pre-dip position to rinse the needle where this is supported by the software. ***Note:*** *Do not perform more than 20 regeneration injections from the same position.*
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 may be misleading.*

Hints and tips

Issue	Actions
Limited amount of analyte	Perform initial scouting over a wide range of conditions with a few 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 3 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. Avoid for example high concentrations of $MgCl_2$ with phosphate-based running buffers.

System variations

The approach to regeneration scouting and verification described in these Instructions For Use is not supported directly in the software for all systems. MDL methods for use in Biacore 1000, Biacore 2000, and Biacore 3000 are included in Regeneration Scouting Kit. The methods can also be downloaded from www.gelifesciences.com/biacore.

Ordering information

If larger volumes are needed (e.g., for preparation of buffers at intervals of 0.1 pH unit), 100 mL packages of glycine buffers can be ordered from GE (see table below).

Product name	Content	Order code
Glycine 1.5	1×100 mL	BR-1003-54
Glycine 2.0	1×100 mL	BR-1003-55
Glycine 2.5	1×100 mL	BR-1003-56
Glycine 3.0	1×100 mL	BR-1003-57

For local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
United Kingdom

www.gelifesciences.com/sampleprep

First published May 2003.

GE Healthcare Bio-Sciences AB
Björkgatan 30, 751 84 Uppsala, Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare Bio-Sciences Corp.
100 Results Way, Marlborough, MA 01752, USA

GE Healthcare Dharmacon, Inc.
2650 Crescent Dr., Lafayette, CO 80026, USA

HyClone Laboratories, Inc.
925 W 1800 S, Logan, UT 84321, USA

GE Healthcare Japan Corporation
Sanken Bldg. 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan

22061829 AD 10/2016 a68