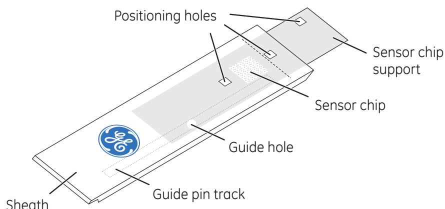
GE Healthcare
Life Sciences

Instruction 22-0519-97 AF Biacore™

Sensor Chip NTA

Product description

Order code:	BR-1004-07 (Package of one sensor chip)
	BR-1000-34 (Package of three sensor chips)
Storage:	The use-before date applies to chips stored at +2 to 8°C in unopened pouches.
Limitation of use:	Use of Sensor Chip NTA as recommended in Biacore 3000 and Biacore 2000 requires that Method Definition Language (MDL) is used. The recommended extra wash is not possible using the software wizards.



Note: *For in vitro use only.*

The sensor chip is fixed to a polystyrene support sheath. Each cassette, consisting of a sensor chip and sheath assembly, is individually packed under a nitrogen atmosphere in a sealed pouch.

Application areas

Sensor Chip NTA is designed to bind histidine-tagged biomolecules for interaction analysis in Biacore systems. The surface consists of a carboxymethylated dextran matrix pre-immobilized with nitrilotriacetic acid (NTA). His-tagged ligands are captured on Sensor Chip NTA by chelation of Ni^{2+} by NTA on the surface and histidine residues in the ligand tag. Other amino acid side chains in the ligand may participate in chelation, but these interactions tend to be weak in comparison to those involving poly-histidine tags.

The affinity of ligand capture varies with the micro-environment around the histidine tag. Optimal binding is obtained with tags consisting of at least 6 histidine residues. Multiple poly-histidine tags on the same ligand can sometimes result in improved stability of capture.

Refer to www.gelifesciences.com/biacore for updates on applications and scientific publications.

Required solutions

See table below for solutions required for use with Sensor Chip NTA.

Solution	Description
Running buffer	HBS-P+ or HBS-P (available from GE Healthcare). Other buffers may be used if they are more appropriate for the interactants being studied. Avoid using buffers containing imidazole or other chelating agents. Some bivalent metal ions such as Ca^{2+}, Zn^{2+} and Cu^{2+} can interfere with binding of Ni^{2+} to NTA. Inclusion of 50 µM EDTA in the running buffer can counteract the effect of contaminant levels of metal ions.
Nickel solution [1]	0.5 mM $NiCl_2$ in water or running buffer. Do not change the $NiCl_2$ concentration unless specifically required.

Solution	Description
Regeneration solution [1]	350 mM EDTA in water or running buffer, pH ~8.3. This solution may be cloudy before final pH adjustment.
Wash solution	3 mM EDTA in water or running buffer. This solution may be prepared by dilution of regeneration solution.

[1] Required Nickel and Regeneration solutions are available as ready-to-use solutions in the NTA Reagent Kit, which may be ordered separately from GE Healthcare (order code: 28-9950-43).

Preparations for use

1. Allow the sealed sensor chip pouch to equilibrate at room temperature for 15 to 30 minutes in order to prevent condensation on the chip surface.
2. Prepare the Biacore instrument with running buffer. The buffer should be filtered (0.22 µm), and degassed for systems that do not have an integrated buffer degasser.
3. Open the sensor chip pouch. Make sure that the sensor chip support remains fully inserted into the sheath at all times.
4. Dock the sensor chip in the instrument as described in the instrument handbook. Sensor chips that are not docked in the instrument should be stored in closed containers.

Analysis cycle

Introduction

The required steps in an analysis cycle are described below. Include at least one start-up cycle in each run before analyzing samples to allow the response to stabilize. Use the same cycle definition for start-up cycles as for samples, including injection of nickel solution and histidine-tagged ligand but with the sample replaced by buffer.

Condition the surface

Immediately before the first analysis cycle in a run, condition the surface with a one-minute pulse of regeneration solution. Include an extra wash (see *Appendix: Sample cycles with extra wash, on page* 8) using running buffer to make sure that all traces of regeneration solution are removed.

Note: *This conditioning has to be done only in the first analysis cycle of each run.*

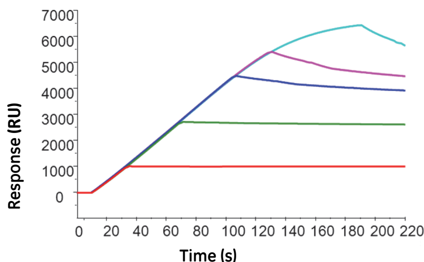
Prepare the surface with nickel

- Inject a one-minute pulse of the nickel solution to saturate the NTA with nickel. Low flow rates (5 to 10 µl/min) can be used. This will result in a small response increase of typically ~40 RU. Include an extra wash (see *Appendix: Sample cycles with extra wash, on page* 8) using wash solution containing 3 mM EDTA after the nickel injection.
- If a blank surface is used as reference, do not inject nickel solution over the reference surface. If you want to use a dummy his-tagged protein on the reference, prepare the reference surface with nickel and capture the dummy protein in the same way as the ligand.

Capture the ligand

- Prepare the histidine-tagged ligand in running buffer. Concentrations below 0.2 µM (30 µg/ml for a protein of molecular weight 150,000) are normally sufficient. If the capture level is too high there is a risk that the ligand may dissociate too fast during the analysis cycle.
- Inject ligand solution over the nickel activated sensor surface with a contact time of typically 1 to 3 minutes. Low flow rates (5 to 10 µl/min) can be used. The capture level is controlled by varying ligand concentration and /or injection time.

If the response after ligand capture is not sufficiently stable, try reducing the amount of captured ligand. Lower ligand levels tend to give more stable capture (see following sensorgram). Alternatively, ligand can be cross-linked after capture as described in *Additional immobilization options, on page 6*. The following sensorgram shows stability for different capture levels, in this case obtained by using different injection times for the ligand.



Inject sample

Interaction analysis is performed as analytes are injected over the ligand captured on the surface of Sensor Chip NTA.

For analysis of crude samples (e.g., cell extracts or culture medium), bear in mind that some non-analyte proteins containing histidine may be able to bind to unoccupied nickel atoms on the surface, resulting in background responses.

Regenerate the surface

Inject regeneration solution for 1 minute. This will remove nickel and any chelated molecules from the surface. Include an extra wash (see *Appendix: Sample cycles with extra wash, on page 8*) with running buffer after the regeneration.

For certain reagents, such as small molecules and fragments, regeneration solution (350 mM EDTA) alone may not be sufficient to regenerate the surface completely. Alternative or extra regeneration injections may be required. Examples of alternative regeneration solutions are:

- 500 mM imidazole (60 s),

  or

- 6 M urea and 350 mM EDTA in 50 mM NaOH (60 to 120 s) (for small molecules)

  Note: *The latter solution should only be used at rack and analysis temperatures above 20°C.*

Injection of either one of the alternative regeneration solutions should be followed by a stabilization time of 60 s.

For detailed information on regeneration strategies, refer to Biacore Sensor Surface Handbook.

Additional immobilization options

Sensor Chip NTA carries unmodified carboxymethyl groups that can be used for covalent immobilization in the same way as for CM-series sensor chips. If ligand capture by chelation is not sufficiently stable, the sensor chip can be activated with EDC/NHS after the nickel injection and before ligand injection to immobilize the ligand covalently by amine coupling. The affinity of the histidine-tagged ligand for the chelated nickel will concentrate the ligand on the surface, often allowing immobilization under physiological buffer conditions. This approach can result in immobilization levels higher than those obtained with nickel chelation alone. Note however that the ligand is permanently attached to the surface, and regeneration conditions appropriate for the specific ligand will need to be established.

Refer to Biacore Sensor Surface Handbook for more information on amine coupling procedures.

Chemical resistance

The surface of Sensor Chip NTA is resistant to many buffers and solutions used in biochemical studies. See table below for information of common agents compatible with Sensor Chip NTA. For information on resistance to other solutions, contact your GE Healthcare representative.

Agent	Concentration
Acetonitrile	30%
DMSO	10%
EDTA	0.35 M
Ethanol	70%
Ethanolamine	1 M
Ethylene glycol	100%
Glycine pH 1.5 to 3.0	100 mM
HCl	100 mM
Imidazole	250 mM
NaOH	100 mM
NaCl	5 M
SDS	0.5%
Surfactant P20	5%
Urea	8 M

References

Refer to Biacore handbooks and www.gelifesciences.com/biacore for details on experimental protocols and methodology.

Appendix: Sample cycles with extra wash

Follow the instructions below to add and run extra washes in sample cycles.

In Biacore X100:

Create a method that includes the following steps:

Step	Description
Surface conditioning	A one minute injection of regeneration solution. Flow rate 10 µl/min is recommended. This step is used only in the first cycle of each run.
Extra wash with buffer	Use the ***Extra wash*** command with running buffer.
Injection of nick-el solution	0.5 mM $NiCl_2$ injected during one minute. Low flow rate (5 to 10 µl/min) can be used. It is recommended to inject $NiCl_2$ over the active surface only, and not over the reference.
Additional wash using wash solu-tion	Use the ***Extra wash*** command with 3 mM EDTA in water or running buffer after injection of $NiCl_2$. This solution does not pass over the surface.
Capture of the ligand	Injection of ligand at concentrations typically < 0.2 µM and during 1 to 3 min. Low flow rate (5 to 10 µl/min) can be used.
Injection of sample	Sample injection, injection times and flow rates depend on the application.
Regeneration	1 to 2 one-minute injections of 350 mM EDTA. Low flow rate (5 to 10 µl/min) can be used.
Extra wash with buffer	Use the ***Extra wash*** command with running buffer.

In Biacore 3000 and Biacore 2000:

- From www.biacore.com/applicationsupporttools - Methods, download the appropriate Method Definition Language (MDL) method
  or
- Create an MDL method that includes the following steps:

Command	Procedure
Surface conditioning	A one-minute injection of regeneration solution. Flow rate 10 µl/min is recommended. This step is used only in the first cycle of each run.
Extra wash with buffer	Use the ***Extraclean*** command.
Injection of nickel solution	0.5 mM $NiCl_2$ injected during one minute. Low flow rate (5 to 10 µl/min) can be used. It is recommended to inject $NiCl_2$ over the active surface only, and not over the reference.
Additional wash with regeneration solution	Wash with a solution of 3 mM EDTA in water or running buffer after injection of $NiCl_2$. This solution does not pass over the surface. Enter the wash commands in the following order: 1 ***WASHPOS n r1a1*** (needle will be washed with wash solution from a defined position). 2 ***WASHPOS s r1a1*** (sample loop will be washed with wash solution from a defined position). 3 ***WASH n*** (needle will be washed with running buffer). 4 ***WASH s*** (sample loop will be washed with running buffer).
Capture of the ligand	Injection of ligand at concentrations typically < 0.2 µM and during 1 to 3 min. Low flow rate (5 to 10 µl/min) can be used.
Injection of sample	Sample injection, injection times and flow rate depend on the application.
Regeneration	1 to 2 one-minute injections of 350 mM EDTA. Low flow rate (5 to 10 µl/min) can be used.
Extra wash with buffer	Use the ***Extraclean*** command.

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

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

www.gelifesciences.com/sampleprep

GE, imagination at work and GE monogram are trademarks of General Electric Company.

Biacore is a trademark of GE Healthcare companies.

© 2008-2013 General Electric Company – All rights reserved.
First published Jun. 2008

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. A copy of these terms and conditions is available on request. Contact your local GE Healthcare representative for the most current information.

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

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

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

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

imagination at work

22-0519-97 AF 03/2013