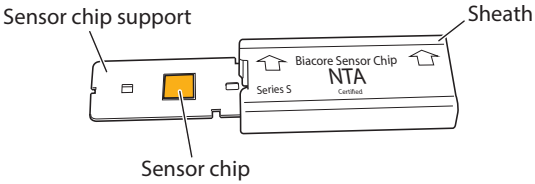
Series S Sensor Chip NTA

Instructions for Use

Product description

Order code:	BR-1005-32 (Package of three sensor chips) 28994951 (Package of one sensor chip)
Storage:	The use-before date applies to chips stored at 2°C to 8°C in unopened pouches.



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

Series S 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). Histidine-tagged ligands are captured on Series S Sensor Chip NTA by chelation of Ni^{2+} through 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. For satisfactory binding, the tags should consist of at least 6 histidine residues. Multiple poly-histidine tags on the same ligand can sometimes result in improved stability of capture.

Refer to *cytiva.com/biacore* for updates on applications and scientific publications.

Required solutions

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

Solution	Description
Running buffer	HBS-P+ or HBS-P (available from Cytiva). 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.
Regeneration solution [1]	350 mM EDTA in water or running buffer, pH ~8.3. This solution may be cloudy before final pH adjustment.
Washing 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 Cytiva (order code: 28995043).

Preparations for use

Step	Action
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 fresh 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. To make sure that all traces of regeneration solution are removed, include a wash using running buffer after the injection.

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

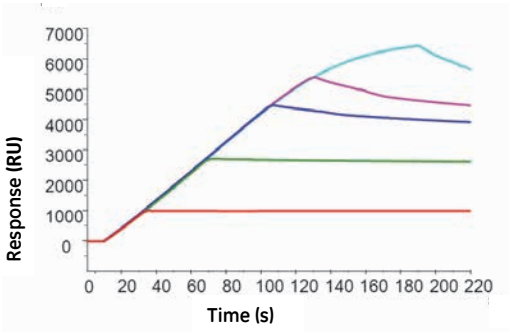
Prepare the surface with nickel

- 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 a wash using running buffer 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 negative control protein on the reference, prepare the reference surface with nickel and capture the negative control 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 *Chapter Additional immobilization options, on page 5*. 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.

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 a wash using 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 will be followed by a stabilization time of 60 s.

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

Additional immobilization options

Series S 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 Series S Sensor Chip NTA is resistant to 1-minute pulses of many commonly used agents. See table below for information of common agents compatible with Series S Sensor Chip NTA.

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

Agent	Concentration
HCl	100 mM
Imidazole	250 mM
NaOH	100 mM
NaCl	5 M
SDS	0.5%
Surfactant P20	5%
Urea	8 M

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

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