NTA Reagent Kit

Instructions for Use

Product description

Product code:	28995043
Contents:	• Nickel solution, 0.5 mM $NiCl_2$, 50 mL (sterile filtered) • Regeneration solution, 350 mM EDTA, 100 mL (sterile filtered)
Storage:	2°C to 8°C
Kit capacity:	The kit contains reagents sufficient for approximately 1200 injections.
Safety:	For use and handling the product in a safe way, refer to the Safety Data Sheet.

Note: *For research use only.*

Intended use

NTA Reagent Kit is intended for use together with Sensor Chip NTA. The kit contains nickel and regeneration (EDTA) solutions, which are necessary for running interaction analysis of histidine-tagged biomolecules on Sensor Chip NTA in Biacore™ systems . Nickel solution creates the chelating surface on Sensor Chip NTA. Regeneration solution regenerates the surface of the sensor chip, and is also used to add EDTA to the running buffer and to prepare washing solution for the instrument system.

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

Preparations for Use

Required materials

See the list below for additional required materials (not provided in the kit):

- Sensor Chip NTA

  Note: *Refer to the Instructions for Use for Sensor Chip NTA*

- Running buffer (HBS-P+)

  Other buffers can be used if they are more suitable for the interactants to be 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, see *Prepare running buffer with EDTA*.

- Washing solution (3 mM EDTA in water or running buffer)

  This solution can be prepared by dilution of regeneration solution, for example 10 mL running buffer + 86 µL regeneration solution.

Prepare running buffer with EDTA

Prepare the running buffer with 50 µM EDTA by adding regeneration solution to the running buffer. The required volume of regeneration solution for preparation of running buffer with 50 µM EDTA are shown in the following table.

Running buffer (mL)	Regeneration solution (µL)
200	29
500	71
1000	143

Recommended running conditions

Analysis temperature

NTA Reagent Kit is designed for use at 25°C. Other temperatures might work but have not been tested.

Start-up cycles

For best assay performance, run at least one start-up cycle using identical settings as for the analysis cycles, including nickel solution and histidine-tagged ligand. Replace the analyte with running buffer.

Sensor chip surface conditioning

Immediately before the first analysis cycle in a run, condition the surface of the sensor chip with a 1-minute pulse of regeneration solution. Include an extra wash using running buffer to make sure that all traces of regeneration solution are removed.

Note: *Conditioning of the sensor chip surface must be done only in the first analysis cycle of each run.*

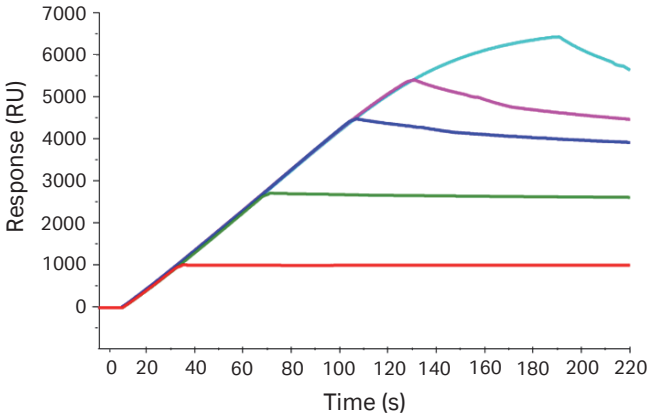
Saturating the sensor chip surface with nickel

- Inject nickel solution with a 1-minute pulse to saturate the sensor chip 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 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 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 injection

- 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 can dissociate too fast during the analysis cycle.
- Inject ligand solution over the nickel-saturated sensor chip 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 5*. The following sensorgram shows stability for different capture levels, in this case obtained by using different injection times for the ligand.



Analyte injection

Use analyte injection conditions appropriate for the assay purpose.

For analysis of crude samples (e.g., cell extracts or culture medium), some non-analyte proteins containing histidines can bind to unoccupied nickel atoms on the surface, resulting in background responses.

Regeneration injection

Inject regeneration solution for 1 minute. This will remove Ni^{2+} and any associated molecules from the surface of the sensor chip. Include an extra wash with running buffer after the regeneration.

For certain reagents, such as small molecules and fragments, 350 mM EDTA alone might not be sufficient to regenerate the sensor chip surface, and alternative or extra regeneration injections can be required. The following are examples of alternative regenerations:

- 500 mM Imidazole (60 s)
- A combination of 6 M Urea, 50 mM NaOH, and 350 mM EDTA (60 to 120 s), followed by a stabilization time of 60 s

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

Refer to *Biacore Sensor Surface Handbook* for detailed information on regeneration strategies.

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, allowing immobilization under physiological buffer conditions. This approach can result in immobilization levels higher than those obtained with nickel chelation alone. Note 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.

For more information on running conditions for different applications, guides, lab protocols, and free eLearnings, visit *cytiva.com/biacore*.

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