GE Healthcare
Life Sciences

Instruction 28-9974-82 AC **Biacore™**

NTA Reagent Kit

Product description

Order code:	28-9950-43
Contents:	• Ni^{2+} solution: 0.5 mM $NiCl_2$, 50 ml (sterile filtered). • Regeneration solution: 350 mM EDTA, 100 ml (sterile filtered).
Storage:	+2 to 8°C
Capacity:	The kit contains reagents sufficient for approximately 1200 injections.
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

NTA Reagent Kit is intended for use together with Sensor Chip NTA. The kit contains solutions necessary for running interaction analysis of histidine-tagged biomolecules on Sensor Chip NTA. The solutions provided are a nickel (Ni^{2+}) solution for creating the chelating surface on Sensor Chip NTA and an EDTA solution for regeneration of the surface. The regeneration solution is also used for addition of EDTA to the running buffer and for preparation of wash solution for the instrument system.

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

Required solutions

Additional solutions

Ni^{2+} solution and Regeneration solution are included in the kit. Additional solutions required are:

- **Running buffer:** HBS-P+ or HBS-P

  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, see *Preparation of running buffer with EDTA* below.

- **Washing solution:** 3 mM EDTA in water or running buffer.

  This solution may be prepared by dilution of regeneration solution, for example 10 ml running buffer + 86 µl regeneration solution.

Preparation of 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 presented in the following table.

Running buffer (ml)	Regeneration solution (µl)
200	29
500	71
1000	143

Preparations for use

Follow the instructions below to prepare Sensor Chip NTA before a run.

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 1-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.*

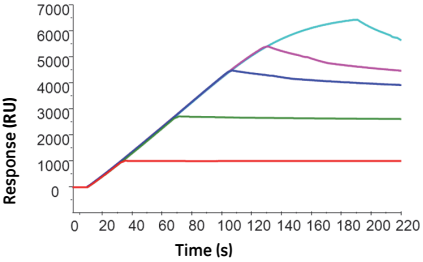
Prepare the surface with nickel

- 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 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 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 7*. 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 histidines 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 Ni^{2+} and any associated 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, 350 mM EDTA alone may not be sufficient to regenerate the surface completely and alternative or extra regeneration injections are required. Examples of alternative regeneration solutions are 500 mM Imidazole (60 s) and, for small molecules, a combination of 6 M Urea, 50 mM NaOH and 350 mM EDTA (60-120 s), followed by a stabilization time of 60 s.

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

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

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 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 using wash solution	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.

In other Biacore systems:

Use the ***Extra wash*** command.

For local office contact information, visit
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