Procedure

Capture of histidine-tagged molecules to Biacore Sensor Chip NTA

This guideline provides recommendations for nickel (Ni^{2+})-mediated capture of histidine-tagged molecules (ligands) to Biacore™ Sensor Chip NTA. Sensor Chip NTA is pre-immobilized with nitrilotriacetic acid (NTA) for capture of histidine-tagged ligands via Ni^{2+}/NTA chelation (*see* Fig 1).

By using Sensor Chip NTA, orientation of the histidine-tagged ligands can be controlled, and the ligand can be captured under physiological conditions. With the regeneration solution included in the NTA Reagent Kit from Cytiva, the sensor chip surface can easily be regenerated and reused.

The ligand capture to Sensor Chip NTA can be stabilized by covalent coupling of the ligands to the chip surface using amine coupling. This will however permanently immobilize the ligand and suitable regeneration conditions for the assay then need to be determined.

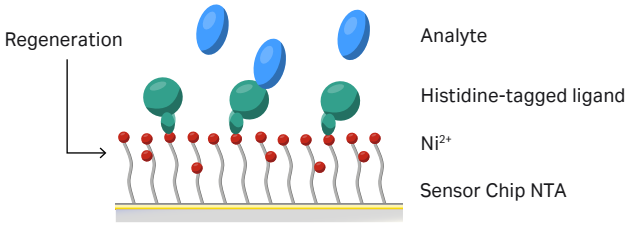

Fig 1. Schematic view of the chip surface of Sensor Chip NTA with immobilized nitrilotriacetic acid (NTA) saturated with nickel (Ni^{2+}), histidine-tagged ligands chelated to Ni^{2+} and analyte binding to the histidine-tagged ligands. The arrow indicates where the regeneration will act on the sensor chip surface.

Ni^{2+}-mediated capture

Preparation

Condition Sensor Chip NTA by injecting regeneration solution (350 mM EDTA in running buffer or water, pH ~ 8.3) over both active and reference surface for 60 s. Include an ***Extra Wash*** command with running buffer to get rid of remaining traces of EDTA. Use flow rate 30 µL/min.

Control cycle

A control cycle to test for unspecific binding of the analyte to nickel before starting the analysis is recommended. The control cycle should be run as a separate experiment. In Biacore systems with more than two flow cells, the control cycle can be run in parallel with the assay.

Include at least one start-up cycle to allow the response to stabilize. Run the start-up and control cycle over the active surface. Use identical settings as for the analysis cycle but exclude injection of histidine tagged ligand. If the analyte shows high nonspecific binding to nickel, it might not be suitable for use with Sensor Chip NTA. Instead, try capturing the ligand to an anti-histidine antibody using His Capture Kit.

Analysis cycle

Include at least one start-up cycle before analysing samples to allow the response to stabilize. Run the start-up cycle over both active and reference surface. Use identical cycle settings as for the analysis cycles, including injection of nickel solution and histidine-tagged ligand, but with running buffer instead of analyte. Nickel solution and regeneration solution are included in NTA Reagent Kit.

1. Saturate the NTA with nickel by injecting nickel solution (0.5 mM $NiCl_2$ in running buffer or water) for 60 s. Include an ***Extra Wash*** command with running buffer containing 3 mM EDTA to remove any remaining traces of nickel in the system. Low flow rates (5–10 µL/min) are recommended.
   - Using a blank surface as reference is recommended. Do not inject nickel solution over the blank reference surface. If using a negative control protein on the reference surface, saturate the reference surface with nickel and capture the negative control protein in the same way as the ligand.
2. Capture the ligand by injecting ligand solution over the nickel-activated sensor surface with a contact time of typically 1 to 3 min. Low flow rates (5–10 µL/min) can be used to reduce ligand consumption.
   - The capture level is controlled by varying ligand concentration and/or injection time.
   - Prepare the ligand in running buffer. Concentrations below 0.2 µM (30 µg/mL for a protein of mol. weight [M_r] 150 000) are normally enough.
3. Perform the interaction analysis by injecting the analyte over the ligands captured on the surface. Recommended starting settings are contact time 120 s and flow rate 30 µL/min.
4. Regenerate the surface by injecting regeneration solution (350 mM EDTA in running buffer or water, pH ~ 8.3) for 60 s. This will remove nickel and any chelated molecules from the surface. Include an ***Extra Wash*** command with running buffer after regeneration to remove remaining traces of EDTA. Use flow rate 30 µL/min.
   - If the regeneration is unsatisfactory, include a second regeneration step (with e.g., 50 mM NaOH for proteins or 10% DMSO for low molecular weight molecules). Use the same contact time and flow rate as for regeneration with EDTA and move the ***Extra Wash*** command to after the second regeneration.

Important considerations

- Always use fresh filtered (0.22 µm) and degassed running buffer.
- The affinity of ligand capture varies with the micro-environment around the histidine tag.
- If the response after ligand capture is not stable, try reducing the amount of captured ligand. Alternatively, the ligand can be covalently immobilized after capture by amine coupling.

Stabilization by amine coupling

Preparation

Condition Sensor Chip NTA by injecting regeneration solution (350 mM EDTA in running buffer or water, pH ~ 8.3) over both active and reference surface for 60 s. Include an ***Extra Wash*** command with running buffer to remove remaining traces of EDTA. Use a flow rate of 30 µL/min.

Immobilization

EDC, NHS, and ethanolamine are available in the Amine Coupling Kit.

1. Saturate the NTA with nickel by injecting nickel solution (0.5 mM $NiCl_2$ in running buffer or water) over the active surface for 60 s. Include an ***Extra Wash*** command with running buffer containing 3 mM EDTA to remove any remaining traces of nickel in the system. Use a flow rate of between 5 and 10 µL/min.
   - Using a blank surface as reference is recommended. Do not inject nickel solution over the blank reference surface. If using a negative control protein on the reference surface, saturate the reference surface with nickel and capture the negative control protein in the same way as the ligand.
2. Activate the surface by injecting EDC/NHS for 7 min using a flow rate between 5 and 10 µL/min.
3. Immobilize the ligand by injecting ligand solution over the activated sensor surface with a contact time of typically 7 min. Use a flow rate of 10 µL/min.
   - Prepare the ligand in running buffer. Concentrations between 5 and 50 µg/mL are normally enough.
4. Deactivate the surface by injection of ethanolamine for 7 min. Use a flow rate between 5 and 10 µL/min.
5. Inject regeneration solution (350 mM EDTA in running buffer or water, pH ~ 8.3) to wash away nickel and any chelated molecules from the surface. Use a contact time of 60 s and flow rate 30 µL/min. Include an ***Extra Wash*** command with running buffer to remove remaining traces of EDTA. Use a flow rate of 30 µL/min.

Analysis cycle

Include at least one start-up cycle before analysing samples to allow the response to stabilize. Run the start-up cycle over both active and reference surface. Use identical cycle settings as for the analysis cycles but with running buffer instead of analyte. Using a blank surface as reference is recommended.

1. Perform the interaction analysis by injecting the analyte over the ligands immobilized on the surface. Recommended starting settings are contact time 120 s and flow rate 30 µL/min.
2. Regenerate the surface by injecting a suitable regeneration solution. For detailed information on regeneration strategies refer to Sensor Surface Handbook. Include an ***Extra Wash*** command with running buffer after regeneration to get rid of remaining traces of the regeneration solution.

Important considerations

- Neutral buffers at physiological ionic strength can be used throughout the immobilization, since initial attachment of the ligand relies on chelation rather than electrostatic preconcentration.
- If the immobilized level is too high, for example, if the surface is intended for kinetic analysis, use a lower concentration of ligand and/or a shorter contact time. Changing the EDC/NHS conditions will have little effect on the immobilized level.

Ordering information

Product	Product code
Sensor Chip NTA, 1 sensor chip	BR100407
Sensor Chip NTA, 3 sensor chips	BR100034
Series S Sensor Chip NTA, 1 sensor chip	28994951
Series S Sensor Chip NTA, 3 sensor chips	BR100532
Amine Coupling Kit	BR100050
NTA Reagent Kit	28995043
His Capture Kit	28995056

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