NTA Sensors

Tech Guide

Immobilization method:	Capture
Ligand requirements:	Histidine Tag (eg. 6x Histidine tag on N-terminus)
Ligand orientation:	Oriented
Recommended coupling kit:	NTA Reagent Kit

Overview

Nicoya's NTA Sensors have a uniform layer of nitrilotriacetic acid (NTA) groups for capture of ligands containing poly-histidine (His) tags. His-tags are commonly used for protein purification and are a convenient method for immobilization. The NTA groups on the sensor surface are activated with Ni^{2+} ions to immobilize the ligand via the His-tag, providing an orientation-specific capture (Figure 1). The NTA Reagent Kit provides EDTA to wash off contaminants bound to the NTA surface, $NiCl_2$ for surface activation, and imidazole to disrupt His-NTA bonds for surface regeneration.

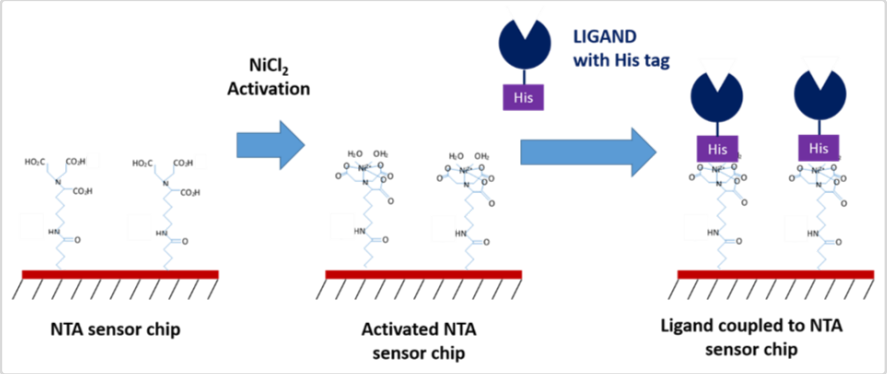

Figure 1. Immobilization of His-tagged ligand onto an NTA Sensor.

Sensor storage buffer	HBS
Recommended storage	4 °C
Shelf life	6 months

Materials and Reagents Required for Coupling:

- **NTA Sensor**
- **NTA Reagent Kit**
  - $NiCl_2$
  - Imidazole
  - EDTA
- **10 mM HCl**

Injection Volumes

Minimum recommended injection volumes recommended for a 100 µL sample loop:

OpenSPR Rev 4	150 µL
OpenSPR-XT Rev 4	200 µL
OpenSPR Rev 3	200 µL
OpenSPR-XT Rev3	300 µL

Buffer Conditions

Conditions to avoid:

- Strong reducing agents (e.g. TCEP, DTT)
- Chelating agents (e.g. EDTA)

Running buffers containing chelating agents will remove the necessary Ni^{2+} ions and reducing agents will alter the Ni^{2+} redox state, both of which may compromise the NTA surface activation

Ligand Removal

It is possible to remove the His-tagged ligand from the sensor surface by disrupting the His-NTA bond. A typical protocol uses 1-2 injections of 500 mM imidazole to remove the ligand from the sensor. Solutions of EDTA (350 mM) and/or acidic conditions (10 mM Glycine-HCl or HCl) can also be used if imidazole does not fully remove the ligand from the sensor.

It is important to take these conditions into consideration when the user is screening for regeneration condition between the analyte and ligand. For example, if an acidic solution is needed for analyte regeneration, but also removes the ligand from the NTA sensor surface, the ligand should be re-coated prior to the analysis of the next analyte concentration.

Referencing

For the 2-Channel OpenSPR, it is recommended to immobilize the ligand in channel 2 only and use a blocked sensor surface (with an inactive His-tagged protein) in channel 1 as the reference. For a non-specific binding experiment using the 1-Channel OpenSPR, it is recommended to prepare a blocked sensor surface (with an inactive His-tagged protein) as a negative control. This will shield the charges of the Ni^{2+} and NTA groups, which can contribute to electrostatic based non-specific interactions of the analyte. Next, inject an analyte at the highest concentration to be used for the experiment. Immobilization of the ligand can be performed on this surface thereafter by removal of the blocking protein.

Additional Notes

The NTA-His ligand capture method is not as strong as a covalent capture technique. The NTA-His binding will have a slow dissociation associated with it, characterized by a reduction in the baseline (negative slope) over time. Because of this inherent dissociation of the captured ligand, this immobilization method is not recommended for analysis of kinetic systems with slow dissociation.

Coupling Procedure

1. Surface Conditioning 1

Perform an injection of 10 mM HCl to clean the sensor surface.

CH	Flow Rate
1+2	150 µL/min

2. Surface Conditioning 2

Perform an injection of 350 mM EDTA to clean the sensor surface.

CH	Flow Rate
1+2	100 µL/min

3. Surface Activation

Perform an injection of 40 mM $NiCl_2$ solution to activate the NTA surface with Ni^{2+} ions. (5 minute interaction time).

CH	Flow Rate
1+2	20 µL/min

4. Ligand Immobilization

Dilute the His-tagged ligand to be immobilized in the running buffer to a concentration of 10-50 µg/mL. Inject the ligand solution into the instrument. (5 minute interaction time).

CH	Flow Rate
2	20 µL/min

5. Evaluation 1

The amount of ligand binding is calculated by comparing the signal after the $NiCl_2$ injection to the signal after the ligand immobilization step. In the example shown in Figure 2, it is approximately 4000 RU. Ensure this meets your minimum ligand immobilization target.

If your immobilization target is not reached, repeat another ligand immobilization injection, or consider optimization of this step.

6. Blocking

Inject a His tagged inactive protein (a protein of similar molecular weight but unable to interact with your analyte) to block the remaining open sites on the sensor. (5 minute interaction time). This step is optional, but highly recommended to reduce NSB in channel 1.

CH	Flow Rate
1+2	20 µL/min

7. Evaluation 2

The amount of blocking protein binding is calculated by comparing the signal before this injection to the signal after the blocking step. In the example shown in Figure 2, it is approximately 2500 RU.

If your immobilization target is not reached, repeat another blocking injection.

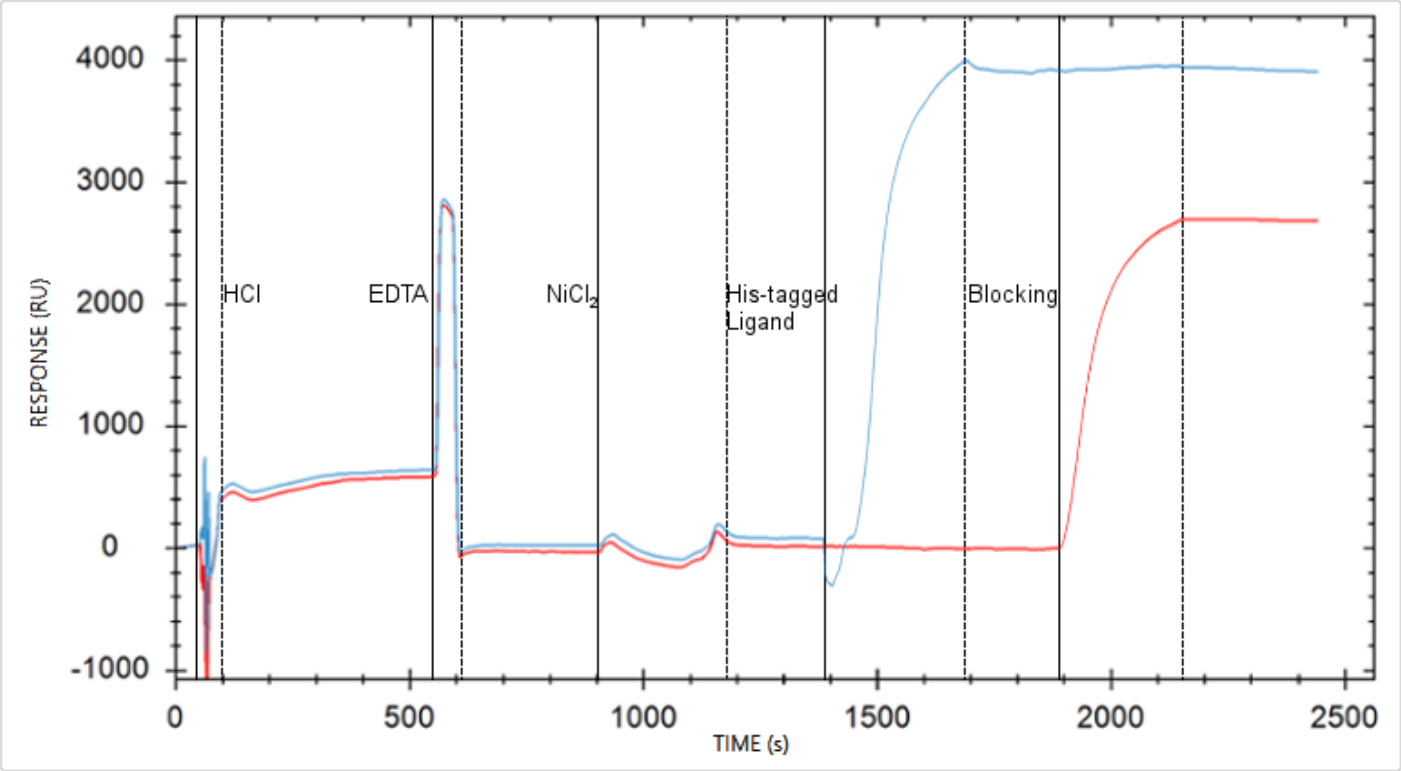

Figure 2. Example of NTA Sensor his-tagged protein immobilization on the 2-Channel OpenSPR system (red: Channel 1, blue: Channel 2).