NTA Sensors

Tech Guide

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

Overview

The NTA Sensors have a uniform layer of nitrilotriacetic acid (NTA) groups for capture of ligands containing poly-histidine (His-6) 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 the $NiCl_2$ needed for surface activation, along with imidazole used to disrupt His-NTA bonds for surface regeneration.

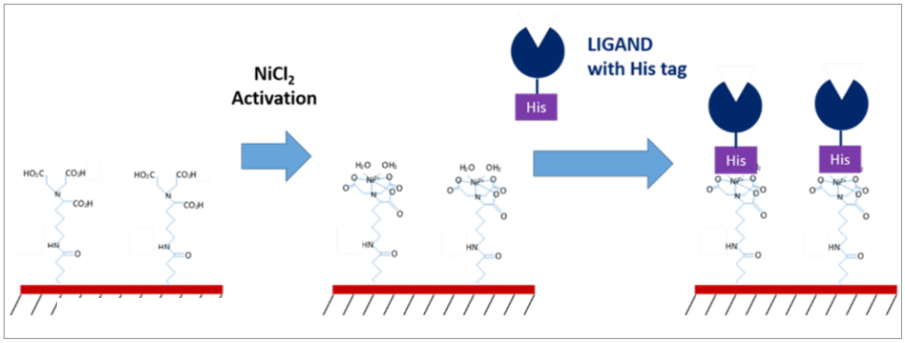

Figure 1. Immobilization of His-tagged ligand onto an NTA Sensor.

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

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. Solutions of imidazole (200 mM), acidic conditions (10 mM Glycine-HCl or HCl), and EDTA can be used to remove the ligand from the sensor. A typical protocol includes an injection of HCl followed by injection(s) of imidazole (sometimes multiple injections may be necessary for full removal).

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

Perform 1-2 injections of 200 mM imidazole to clean the sensor surface.

CH	Flow Rate
1+2	150 µL/min

2. 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

3. Ligand Immobilization

Dilute the His-tagged ligand to be immobilized in 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

Evaluation

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 6000 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.

4. Blocking

Inject a His-tagged negative control 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)

CH	Flow Rate
1+2	20 µL/min

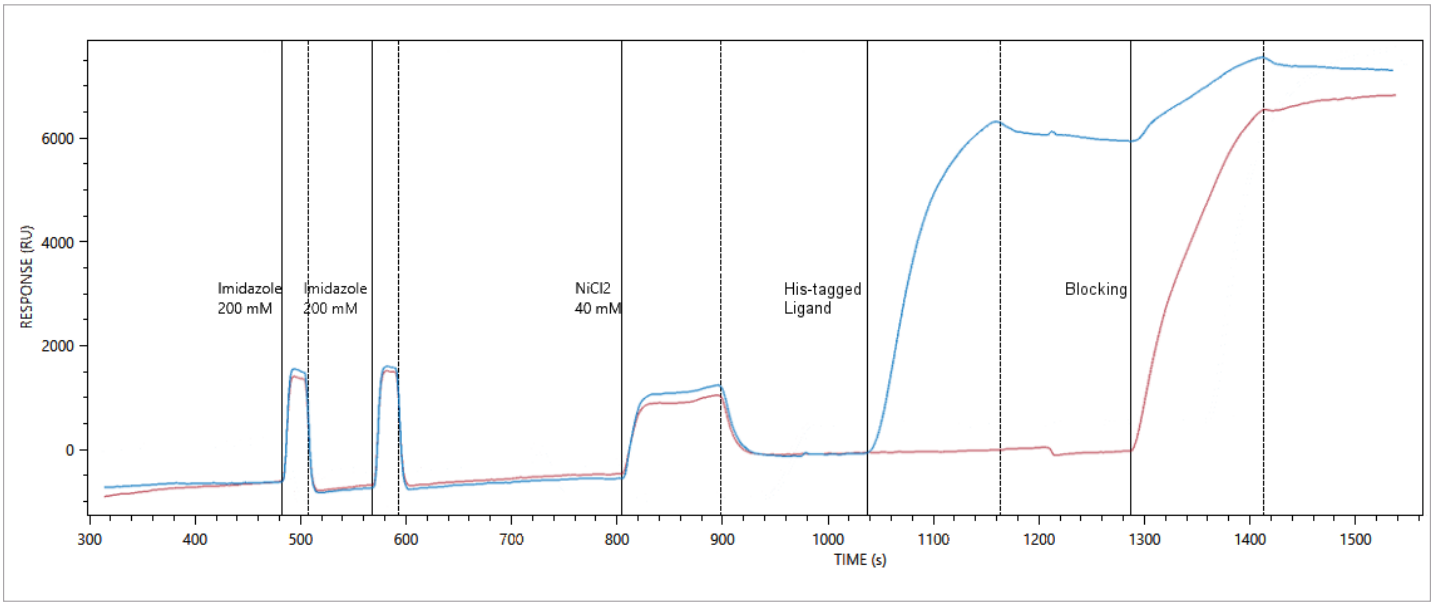

Figure 2. Example of NTA Sensor His-tagged protein immobilization on the 2-Channel OpenSPR system (red: Channel 1, blue: Channel 2).