Octet® NTA Biosensor
Kinetic Assays

Abstract

The Octet® NTA Biosensor is pre-immobilized with novel nickel-charged tris-nitrilotriacetic (Tris-NTA) groups for quick and easy capture of purified HIS-tagged molecules for the kinetics characterization. The biosensor surface is designed as an alternative to chemical ligand immobilization protocols such as EDC/NHS and biotinylation; however, in cases where surface stability needs improvement, the captured HIS-containing ligand can be stabilized by the chemical cross-linker EDC/NHS. NTA Biosensors can be regenerated using low pH glycine buffer while recharging with Ni²⁺ ions.

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Overview

A polyhistidine tag (also known as hexa histidine-tag, 6xHIS-tag, or by the trademarked name HIS-tag) is commonly fused to recombinant proteins to facilitate their detection and purification. The polyhistidine sequence exhibits strong binding to nickel (Ni\textsuperscript{2+}). The NTA Biosensor is pre-immobilized with novel nickel-charged tris-nitrilotriacetic (Tris-NTA) groups for quick and easy capture of HIS-tagged molecules. In conjunction with Octet\textsuperscript{®} Bio-Layer Interferometry (BLI) systems, the NTA Biosensor provides a rapid and label-free method for HIS-tagged protein quantitation and kinetic analysis. For more information on quantitation analyses using the NTA Biosensor, please see the Octet\textsuperscript{®} NTA Biosensor Quantitation Assays Technical Note.

Principle

QIAGEN’s Tris-NTA is charged with nickel (Ni\textsuperscript{2+}) and pre-immobilized onto the biosensor and will bind specifically to a HIS-tag attached to recombinant proteins. The Ni\textsuperscript{2+} captures HIS-tagged analytes for both quantitation and kinetic applications. The binding of the target analyte to the immobilized HIS fusion proteins alters the interference pattern of light reflected from the biosensor surface, allowing molecular association and dissociation events to be monitored in real time using the Octet\textsuperscript{®} BLI platform. The surface is well suited for capture and analysis directly from complex mixtures as an alternative to chemical protocols such as EDC/NHS and biotinylation.

NTA Biosensors can be regenerated up to 10 times via a standard low-pH protocol in as little as two minutes for various applications such as acquisition of replicate data (same ligand/analyte pair) and bucket-based screening applications. Regeneration dissociates the HIS-tagged protein from the biosensor surface, allowing additional analyses. Once the biosensor is regenerated, it must be recharged with Ni\textsuperscript{2+} prior to loading the next HIS-containing proteins. For the highest quality affinity and unique capture ligand is recommended.

Materials Required

- Octet\textsuperscript{®} BLI system with Octet\textsuperscript{®} Software
- NTA Biosensors (Sartorius part no. 18-5101 [tray]; 18-5102 [pack]; 18-5103 [case])
- For all Octet\textsuperscript{®} instruments: 96-well, black, flat bottom, polypropylene microplate (Greiner Bio-One part no. 655209) or Sartorius recommended sample plates
- Optional for Octet\textsuperscript{®} RH16 and RH96 BLI systems:
  - 384-tilted well, black, flat bottom, polypropylene microplate (Sartorius part no. 18-5080 [pack]; 18-5076 [case])
  - 384-well, black, flat bottom, polypropylene microplate (Greiner Bio-One part no. 781209)
- HIS-containing protein for immobilization. HIS-containing protein can be present in either buffer or a complex mixture such as culture supernatant.
- Analyte proteins that interact with HIS-fusion protein. The analyte proteins can be present in buffer matrix or a complex mixture such as culture supernatant.
- Kinetics Buffer. The NTA Biosensor is compatible with a wide range of buffers although 1X Kinetics Buffer is recommended (dilute 10X Kinetics Buffer, Sartorius part no. 18-1042, 1:10 with PBS, pH 7.4). Best results are obtained when samples are free of chelating agents (e.g., EDTA, imidazole, etc.) and all matrices are matched as closely as possible.

Figure 1

*Example Workflow of a Kinetics Characterization Using Octet\textsuperscript{®} NTA Biosensors.*

Note. Example workflow for kinetic characterization of the interaction between a HIS-fusion protein and a target analyte. The assay consists of five assay steps. Step 1: equilibration, Step 2: loading (capture) of HIS-fusion Protein, Step 3: baseline, Step 4: association, Step 5: dissociation.
- Optional:
  - Regeneration buffer (10 mM glycine, pH 1.7): required for surface regeneration. Further evaluation of the most appropriate pH and optimization may be required.
  - Re-charging reagent (10 mM NiCl₂ in H₂O): required after surface regeneration.
  - Cross-linking reagent (0.1 M EDC + 0.025 M NHS in H₂O): used to stabilize the captured protein. Further evaluation and optimization may be required.
  - Quenching reagent (1 M ethanolamine, pH 8.5): used after the cross-linking step.

- Turn on the Octet® BLI system at least 40 minutes before starting the assay, allowing the lamp to warm up.
- Set the sample plate temperature in the Octet® Software by selecting **File > Experiment > Set plate temperature** and entering the desired temperature. Sartorius recommends assaying at 30°C. Using other temperatures may require modifying the assay times discussed in this protocol.

**Assay Protocol**

**Overview**

1. Prepare the Octet® BLI system and assay solutions.
2. Prepare the sample plate and biosensor hydration assembly; equilibrate both 10 minutes on the instrument.
3. Run the assay.
4. Process and analyze the data.
5. Save the results.

**Prepare the Octet® BLI System and Assay Solutions**

Note: Equilibrate reagents and samples to room temperature prior to preparation and mix thoroughly.

1. Ensure that the system and computer are turned on. It is essential that the instrument lamp warms up for at least 40 minutes before running an experiment.
2. **HIS-containing ligand:** The ligand is the protein that will be immobilized on the biosensor tip surface. HIS-containing ligands are typically immobilized at a concentration between 5–25 μg/mL. If the concentration of the HIS-containing ligand is below 5 μg/mL, a longer loading time may be required (60 minutes loading at 1 μg/mL is equivalent to 10 minutes loading at 10 μg/mL). The ligand solution can be recovered from the well after the assay and re-used, if desired. If the ligand is captured from a cell culture supernatant, dilution of the supernatant ten-fold or greater with 1X Kinetics Buffer can potentially increase data quality. If dilution results in a low total concentration of the ligand, the biosensors can be incubated in the diluted supernatant overnight at 4°C to maximize loading of the HIS-containing ligand. For tips on optimizing overnight loading, see the Sartorius Technical Note, Batch Immobilization of a Biotinylated Ligand onto Streptavidin Biosensors.
3. **Interacting protein (analyte):** During rigorous kinetic analysis, it is recommended to run a dilution series of at least four concentrations of the analyte protein. The highest concentration should be approximately 10 times the expected $K_D$. For example, concentrations of 90 nM, 30 nM, 10 nM and 3 nM would be recommended for an analyte with low-nanomolar affinity towards an immobilized ligand. 200 µL/well, 80 µL/well and 40 µL/well of analyte solution are required for 96-well, 384-well, and 384-well tilted-well plates, respectively. The solution can be recovered from the well after the assay and re-used, if desired. For screening assays or qualitative interaction analysis, a single concentration of the interacting protein can be sufficient to characterize the binding.

4. **Regeneration solution:** The captured HIS-containing ligand and the analyte can be removed from the biosensors by exposing them to 10 mM glycine pH 1.7 followed by a neutralization buffer (typically 1X Kinetics Buffer). 200 µL/well, 80 µL/well and 40 µL/well of regeneration and neutralization solutions are required for 96-well, 384-well, and 384-well tilted-well plates, respectively. After regeneration, the biosensor can be re-charged with 10 mM NiCl$_2$ for 1 minute and then re-loaded with HIS-containing protein for a new interaction analysis. A small loss in binding capacity after a regeneration cycle is expected. Regeneration provides a cost-effective method of generating replicate data for ligand-analyte pairs. For the highest quality kinetic results, using a new biosensor to capture each unique ligand is recommended.

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**Figure 2**

**Example Sample Plate Map.**

*Note.* Example plate map for a kinetic assay that includes equilibration, ligand loading, baseline, association, and dissociation steps. Table 1 shows an assay step list for this sample plate. Column 3 is used for both the baseline and dissociation steps to make optimal use of the inter-step correction processing feature.

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**Table 1**

**Example Assay Workflow Steps.**

<table>
<thead>
<tr>
<th>Step</th>
<th>Step name</th>
<th>Time (s)</th>
<th>Shake Speed</th>
<th>Step Type</th>
<th>Column</th>
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<tr>
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<td>Baseline 1</td>
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<td>1000</td>
<td>Baseline</td>
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</tr>
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<td>2</td>
<td>HIS-fusion Protein</td>
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<td>1000</td>
<td>Loading</td>
<td>2</td>
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<td>3</td>
<td>Baseline 2</td>
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<td>1000</td>
<td>Association</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Dissociation</td>
<td>600–1800</td>
<td>1000</td>
<td>Dissociation</td>
<td>3</td>
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</table>

*Note.* Example assay steps and associated parameters. The same wells are used for the baseline and dissociation steps to make optimal use of the inter-step correction processing feature.
Table 2

Example Assay Workflow With Cross-Linking Step.

<table>
<thead>
<tr>
<th>Step</th>
<th>Step Name</th>
<th>Time (s)</th>
<th>Shake Speed</th>
<th>Step Type</th>
<th>Column</th>
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</thead>
<tbody>
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<td>1000</td>
<td>Baseline</td>
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<tr>
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<td>Quenching</td>
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<tr>
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<td>Dissociation</td>
<td>600–1800</td>
<td>1000</td>
<td>Dissociation</td>
<td>5</td>
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</table>

Note. Example assay steps and associated parameters. The same wells are used for the baseline and dissociation steps to make optimal use of the inter-step correction processing feature.
Running the Assay

1. Set up a kinetic assay. For details, see the Octet® Software User Guide. Table 1 shows an example kinetic assay consisting of equilibration, ligand loading, baseline, association, and dissociation. Figure 2 shows an example plate map for a typical kinetic assay. Table 2 and Figure 3 show an example plate map and stepwise procedure for a kinetic assay with cross-linking of the HIS-tagged protein after loading. Table 3 and Figure 4 show an example plate map and stepwise procedure for a kinetic assay with sensor regeneration and recharging.

2. Run the assay.

Process and Analyze the Data

1. Load data into the Octet® Analysis Studio Software.
2. Process the data by specifying methods for reference subtraction, y-axis alignment, inter-step correction and Savitzky-Golay filtering. For details on each processing parameter, refer to the Octet® Software User Guide.
3. Analyze the data by specifying steps for analysis, fitting method (local or global) and window of interest. For details on each analysis parameter, refer to the Octet® Software User Guide.
4. To export the analyzed data, use the Save Report button to generate a Microsoft® Excel report®. For details on data exporting, refer to the Octet® Software User Guide.

Representative data

Figure 5 shows kinetic analysis of the interaction between HIS-endostatin and its interaction partner anti-endostatin. NTA Biosensors were hydrated for 10 minutes in 1X Kinetics Buffer prior to analysis. Assay steps included: 5 minutes of equilibration, 5 minutes of HIS-endostatin loading (20 µg/mL), 5 minutes of baseline stabilization, 15 minutes of HIS-endostatin : anti-endostatin association and 15 minutes of HIS-endostatin : anti-endostatin dissociation. Analyte concentrations were 1.11, 3.33, 10, and 30 nM. 1X Kinetics Buffer was used as the matrix throughout and the assay temperature was 30°C. Data were processed and curve fit using a 1:1 binding model. The kinetic results are reported in Table 4.

Table 4

<table>
<thead>
<tr>
<th></th>
<th>( k_{on} ) (1/M s)</th>
<th>( k_{off} ) (1/s)</th>
<th>( K_D ) (nM)</th>
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</thead>
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<tr>
<td></td>
<td>8.1x10^4</td>
<td>1.8x10^5</td>
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</table>

Note. Kinetic analysis of the interaction between HIS-endostatin and anti-endostatin using NTA Biosensors.