SARTORIUS

Octet® NTA Biosensor Quantitation Assays

Technical Note

Abstract

The Octet® NTA Biosensor is pre-immobilized with novel nickel-charged tris-nitrilotriacetic (Tris-NTA) groups for quick and easy binding of purified HIS-tagged molecules. The NTA Biosensor provides a rapid and label-free method for HIS-tagged protein quantitation. A calibration standard protein identical to the His-tagged protein in the unknown sample is required for the accurate determination of the unknown sample's concentration. The biosensor has a dynamic range of 0.5 – 1000 µg/mL.

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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 (Ni2+). 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 Bio-Layer Interferometry (BLI) platforms such as Octet® BLI systems, the NTA Biosensor provides a rapid and label-free method for HIS-tagged protein quantitation and kinetic analysis. For more information on kinetic analyses using the NTA Biosensor, please see the NTA Biosensor Kinetic Assays Technical Note.

Principle

QIAGEN's Tris-NTA is charged with nickel (Ni2+) and pre-immobilized onto the biosensor and will bind specifically to a HIS-tag attached to recombinant proteins (refer to the QIAGEN QIAexpress Handbook for more information). This binding is monitored in real time using an Octet® BLI system and can be compared to binding a known calibrator to determine concentration. With appropriate dilution, it is possible to measure analytes in complex matrix conditions (see Table 1). The concentration is calculated based on the binding kinetics of the HIS-tagged protein, therefore best results are achieved when the protein used for calibration and the unknown samples are identical. The use of Octet® NTA Biosensor also requires that the samples and buffers be free from EDTA, imidazole, or other similar chelating agents.

Materials Required

- Octet® instrument with Octet® Software
- Octet® NTA Biosensor (Sartorius part no. 18-5101 [tray]; 18-5102 [pack]; 18-5103 [case])
- For all Octet® instruments: 96-well, black, flat bottom, polypropylene microplate (Greiner Bio-One part no 655209) and any other Sartorius recommended sample plates
- Optional for Octet® 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)
- Standard protein containing a HIS-tag, to be used as a calibration standard. For best results this calibration standard protein should be identical to the protein in the sample
- Sample Diluent (Sartorius part no. 18-1000) for dilution of all samples

Tips for Optimal Performance

- HIS-tagged analytes possess different binding kinetics due to amino acid sequence variations and differing steric environments. Since quantitation performance is typically based on binding kinetics, for best results the calibration standard protein should be identical to the HIS-tagged protein in the unknown sample.
- Typical assay sensitivity ranges from 0.5–1000 µg/mL for assays run at 1000 rpm with a 2-minute read time. If a higher-sensitivity assay is needed, the assay time can be extended to 5 minutes.
- Match the matrix of the samples, standards, references, and hydration solution as closely as possible.
- Perform a dilution study and a dynamic range study as outlined in the Assay Optimization section.
- Use a blank negative control in a matching matrix for reference subtraction. This is especially important when optimizing accuracy and detecting low-concentration analytes.
- Fully equilibrate all reagents, calibrators and samples to room temperature prior to sample preparation. Thaw frozen samples completely and mix thoroughly prior to use.
- Hydrate the biosensors for a minimum of 10 minutes prior to use.
- Turn on the Octet® instrument 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 **Experiment > Set Plate Temperature...** and entering the desired temperature. Sartorius recommends 30°C for accurate quantitation. In Octet® Software version 6.4 and later, set the default startup temperature by selecting **File > Options** and entering the desired temperature under Startup.

Assay Optimization

The following optimizations are recommended each time a new matrix or a new HIS-tagged protein is analyzed.

Overview

1. Determine the minimal dilution factor required to achieve the targeted assay performance.
2. Perform a spike/recovery study to determine assay dynamic range.
3. Determine data analysis parameters.
4. Apply finalized protocol and data analysis parameters in routine assay.

Determining the Matrix Dilution Factor

Differences between matrices can potentially influence assay performance. Diluting the sample matrix in Sartorius' Sample Diluent is a convenient and generally effective way to minimize matrix effects. Therefore, it is recommended to determine the minimum dilution factor using Sample Diluent that achieves the desired assay performance.

1. Prepare 2 mL of sample matrix diluted both two-fold and ten-fold in Sample Diluent. General guidelines for dilutions are given in Table 1.
2. Prepare a spiked sample of the HIS-tagged protein to be quantified in: Sample Diluent, neat matrix, two-fold diluted matrix and ten-fold diluted matrix by mixing the minimum volume of HIS-tagged protein and 0.5 mL of each matrix (four samples total). The final concentration of the HIS-tagged protein should be in the middle of the desired quantitation range.
3. Transfer each sample to a 96-well or 384-well sample plate in duplicate (eight wells total).
4. Hydrate biosensors in the sample matrix that matches each sample type (e.g., biosensors to be used in wells with ten-fold diluted matrix should be hydrated in ten-fold diluted matrix). Place the sample plate and the hydrated biosensors in the Octet® BLI system. Recommended sample plate warm-up and biosensor hydration time in the instrument is 10 minutes. The delay timer can be used to automatically start the assay after 600 seconds.
5. Set up a Basic Quantitation assay according to the instructions in the Octet® Software User Guide. Modify Assay Settings to use Standard Assay and set the time to be 120 seconds and shake speed to be 1000rpm.
6. Run the experiment.
7. Data will be displayed in real time during the assay. Data and method files will be saved automatically.
8. Load data into Octet® Analysis Studio Software.
9. Visually inspect the real-time binding traces and determine the dilution required to:
   a. Minimize non-specific binding due to the matrix
   b. Show equivalent HIS analyte binding in the matrix-spiked sample and the Sample Diluent control.
10. Use this dilution factor for routine assays.

Table 1
Minimum Recommended Dilutions.

Sample type	Minimum Recommended Dilution in Sample Diluent
Purified proteins	Dilute into assay range
Samples from column eluents	Dilute into assay range (must be free from EDTA, imidazole, or similar chelating agents)
Serum-free cell culture supernatants	Ten-fold
Serum-containing cell culture supernatants	Ten-fold
Bacterial cell pellet lysed by sonication	Twenty-fold
Bacterial cell pellet lysed by B-PER	One hundred-fold

Note. Minimum recommended dilution for common sample types. In all cases the matrix for the diluted samples, the standards and the biosensor hydration solution should be matched as closely as possible.

Spike Recovery Assay

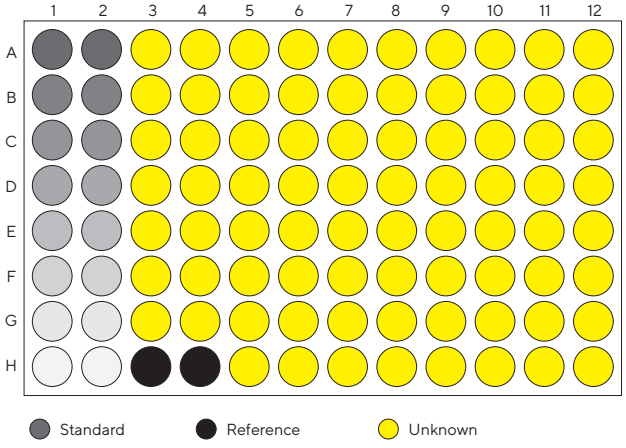
To determine the dynamic range and data analysis parameters suitable for a specific HIS analytes, establish a standard curve and spike recovery as described below.

1. Prepare a series of HIS standards in matrix using the dilution factor determined above. The typical range of the series spans 0.5–1000 µg/mL for the Standard Range Assay at 1000 rpm. A minimum volume of 200 µL/well in a 96-well microplate, 80 µL/well in a 384-well microplate or 40 µL/well in a 384-well tilted bottom microplate is required. Recommended concentrations for the standard curve are described in Figure 2.
2. Using the same sample matrix as in Step 1, prepare 700 µL of two unknown samples. The concentration of these samples should be within the assay dynamic range.
3. Transfer triplicates of the standards and the unknowns to a sample plate. It is recommended to organize samples in columns, from A–H. Fill at least one well with blank diluted matrix for reference subtraction during data analysis. An example plate map is shown in Figure 1.
4. Hydrate biosensors in Sample Diluent or diluted matrix that matches the blank diluted matrix. Place the sample plate and the hydrated biosensors in the Octet® BLI system. The recommended sample plate warm-up in the instrument and biosensor hydration time is 10 minutes. The delay timer can be used to automatically start the assay after 600 seconds.
5. Set up a Basic Quantitation assay using either the Standard or High Sensitivity Assay parameters that were used in the matrix dilution assay above. Availability of the pre-loaded Ni-NTA assay parameters will be software version-dependent.
6. Run the experiment. Data will be displayed in real time during the assay. Data files, method files and assay pictures (.jpgs) will be saved automatically.
7. Load the data into Octet® Analysis Studio Software.
8. If blank matrix was included as a reference, use the reference subtraction option to correct the data as appropriate.
9. Calculate the binding rate.
10. Define a dynamic range by selecting acceptable % CV values for the lower and upper concentration limits. 10% is routinely used as a threshold, but may vary depending on the requirements of each assay.
11. Exclude data points for the standard curve that lie outside the defined dynamic range.
12. Individually adjust the following processing parameters and re-calculate the binding rate:
    a. Adjust the zero concentration threshold if necessary (recommended 0.0001).
    b. Adjust the read time window if necessary (typically 120 seconds).
    c. Adjust the low concentration threshold to 0.003 (recommended).
    d. Select the appropriate standard curve equation.
13. Evaluate the calculated concentration value of the unknowns by defining acceptable values of % recovery (accuracy) and % CV (precision). Frequently used threshold values are ± 15% recovery and 10% CV, but threshold values may vary depending on the requirements of each assay. See Figure 2 and Table 2 for representative data at 1000 rpm.

Assay Protocol

1. Prepare samples, calibration standards and hydration solutions according to the information contained in Table 1. The minimum volume needed in each well varies with the plate used:
   - 200 µL/well in a 96-well microplate (all Octet® BLI systems)
   - 80 µL/well in a 384-well microplate (Octet® RH16 and RH96 BLI systems).
   - 40 µL/well in a 384-well, tilted bottom microplate (Octet® RH16 and RH96 BLI systems).
2. Pipette standards, controls and samples into a black polypropylene microplate (see Figure 1 for a sample plate layout).
3. Pipette biosensor hydration solution into wells of a 96-well black flat bottom microplate corresponding to the number and positions of the biosensors to be used.

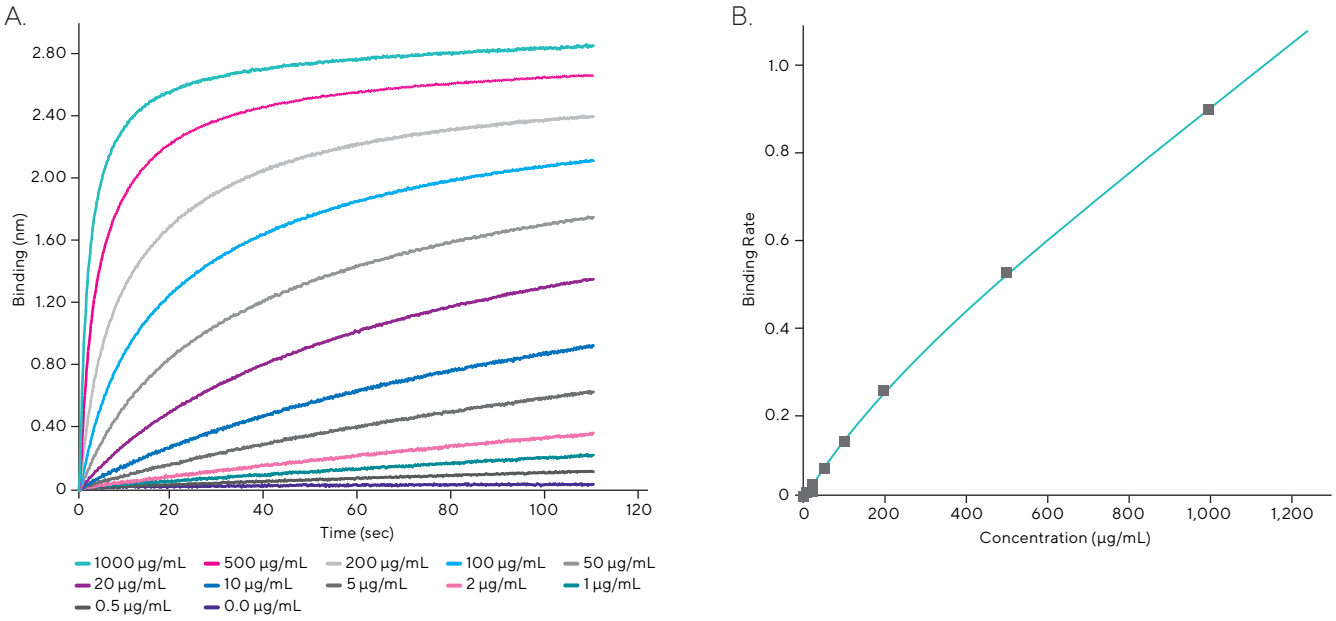
Figure 1
Example Plate Layout.



Note. Example layout for a routine assay run in a 96-well microplate.

Figure 2

Binding Curves of a His-Protein A Standard at Different Concentrations.



Note. Quantitation of a concentration series of a HIS-Protein A standard using NTA Biosensors on an Octet® RH16 BLI system with assay parameters for a standard dynamic range. A) Concentration measurement at 1000 rpm and 2 minutes read time. B) Calibration curve calculated from measuring the standard concentration series. Sample Diluent was used as the matrix for all samples.

4. Place the biosensor tray with the hydration plate in the Octet® BLI system. Place the sample plate in the Octet® BLI system. Warm the sample plate in the instrument and hydrate the biosensors for 10 minutes prior to starting the experiment. The delay timer can be used to automatically start the assay after 10 minutes (600 seconds).
5. Set up a Basic Quantitation assay. An example plate map is shown in Figure 1. For details on how to set up an assay see the Octet® Software User Guide. The dynamic range of the assay can be tuned by changing the shake speed and the read time.
6. Run the assay.
7. Load data into Octet® Analysis Studio Software.
8. If blank matrix was included as a reference, use the reference subtraction option to correct the data as appropriate.
9. Calculate the binding rate.
10. Define a dynamic range by selecting acceptable %CV values for the lower and upper concentration limits. 10% is routinely used as a threshold, but may vary depending on the requirements of each assay.
11. Exclude standard curve data points that lie outside the defined dynamic range.
12. Iteratively adjust the following processing parameters and re-calculate the binding rate:
    a. Adjust the read time window if necessary (typically 120 seconds).
    b. Adjust the zero concentration threshold if necessary (recommended 0.0001).
    c. Adjust the low concentrations threshold to 0.003 (recommended).
    d. Select the appropriate standard curve equation.
13. To export the analyzed data, use the Save Report button to generate a Microsoft® Excel® report.

Representative Data

Figure 2 shows detection of a HIS-Protein A standard using NTA Biosensors on an Octet® RH16 and RH96 BLI systems with assay parameters for a standard dynamic range. A) Assay run at 1000 rpm and 2-minute read time. B) Calibration curve derived from A). Sample Diluent was used as a matrix for all samples. See Table 2 for the statistical analysis of the Figure 2 data.

Table 2

Accuracy and Precision Data for His-Protein A Calibration Standard

Expected Concentration (µg/mL)	Standard Range 1000 rpm 2 min. Read Time	
	Avg. Conc. µg/mL (N = 3)	% CV (N = 3)
1000	1000.00	0.2%
500	500.33	1.0%
200	200.17	0.7%
100	100.07	1.6%
50	50.00	0.5%
25	20.03	0.8%
10	9.97	2.0%
5	5.02	5.2%
2.5	1.99	3.7%
1	1.00	3.2%
0.5	0.50	8.2%

Note. Accuracy and precision for standard curve data. Average calculated concentration and %CV of triplicate HIS-Protein A calibration standards, derived from the data in Figure 2. Results may vary with individual HIS analytes and assay matrices.

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