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Best Practices for Performing Quantitation Assays Using the Octet® N1 System

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Abstract

While ELISA and HPLC are commonly used techniques for determining concentration of a target protein, they are complex and time consuming, especially when analyzing complex matrices. For bioprocess development and production applications, more rapid protein analysis techniques are required to enable timely, informed process decisions.

The Octet® N1 system streamlines workflows and provides rapid, direct quantitation of proteins, even in crude matrices. Proteins and antibodies can be quantitated in seconds with high specificity and sensitivity. The system requires only 4 µL to perform an assay, which allows the user to preserve precious sample when determining affinity, concentration, and binding kinetics.

In this application note, best practices for adopting the Octet® N1 system, along with developing and running quantitation assays, sample and biosensor preparation, and data analysis are provided.

Introduction

The Octet® N1 system provides a simple, rapid Dip and Read approach for protein quantitation in a small, affordably priced personal assay system. With Octet® N1, proteins and antibodies can be quantitated in a matter of seconds with high specificity and sensitivity, even in crude samples. The system utilizes the same proprietary Bio-Layer Interferometry (BLI) technology as Sartorius' Octet® platform, enabling real-time analysis of interactions on the surface of disposable fiber optic biosensors. Affinity, concentration and binding kinetics can be measured right at the bench in a 4 µL drop of sample.

Traditional techniques for determining concentration of a target protein such as ELISA and HPLC are both elaborate and time consuming, especially when analyzing complex matrices. For bioprocess development and production applications, the use of more rapid protein analysis techniques enables timely, informed process decisions. The Octet® N1 system can both streamline workflows and obtain rapid, direct quantitation of proteins in crude matrices.

In this application note, we'll describe best practices for getting started with the Octet® N1 system, as well as developing and running quantitation assays, sample and biosensor preparation, and data analysis.

Getting Started

1. Place the Octet® N1 system in a room with a constant temperature away from AC vents, air fans, heat-generating equipment, and direct sunlight. Do not place the system on the same bench with a centrifuge.
2. When using the Octet® N1 system, all reagents, including the hydration buffer, should be brought to room temperature prior to the start of an experiment.
3. Unused biosensors must be stored inside the original biosensor pouch and sealed with the desiccant pack, and must stay dry. The sealed biosensors should then be stored at room temperature in a dry location. Biosensors cannot be frozen. If a biosensor is ever stored frozen, it loses its activity and cannot be used any more.
4. Turn on the Octet® N1 system and allow it to warm up for at least one hour prior to use.
5. Take note of the temperature of the room, the biosensor lot number, and date to compare data run across multiple days or weeks.
6. Ensure that the drop holder is clean before use.
   a. If the drop holder is still wet, use a Kimwipe® to clean it.
      NOTE: To prevent cross contamination, do not reuse the same part of the Kimwipe to perform another cleaning step.
      i. Add 10–20 µL of sample diluent directly into the drop holder. Do not let the liquid overflow. Pipette up and down five times, then dab off the excess liquid using a Kimwipe. Finally, dry the inside of the drop holder very well with a corner of the Kimwipe using your thumb nail to push the Kimwipe into the drop holder and turn clockwise a few times.
      ii. Repeat the above cleaning step a total of 3–5 times.
      iii. Inspect the dryness of the drop holder under light to ensure it is completely dry.
   b. If samples are allowed to dry out in the drop holder, a solution of 0.5 M HCl can be used to clean it.
      i. Add 10–20 µL of the HCl solution directly into the drop holder. Rub the drop holder with a swab, and then dab off the liquid with a Kimwipe. If excess HCl overflows from the drop holder, wipe off the excess liquid with a Kimwipe.
      ii. Place the drop holder in a 50 mL conical tube with 30–50 mL of purified water. Shake the tube back and forth 5–10 times and discard the used water. Repeat the wash a total of 3 times.
      iii. Next rinse the drop holder with sample diluent, using the steps listed in step a. Do this a total of 5 times.
   c. To confirm cleaning, you can run a Quick Yes/No experiment to verify that no contaminating proteins have been left behind to bind to a biosensor.

Considerations for Developing a Successful Quantitation Assay

1. Standards and unknowns should be of the same protein.
2. All samples measured for quantitation (including standards and unknown samples) should use exactly the same matrix. For example, if the standards in buffer 1 are diluted 10x in buffer 2, unknowns in buffer 1 should also be diluted 10x in buffer 2. This ensures that the final concentration of the bulk components in the final matrix of standards and unknowns should be the same.

3. For the first run, always measure a reference biosensor using buffer only to identify any non-specific binding, matrix or background issues. If a high background signal is observed, the assay should be optimized.
4. Use at least 6–8 known sample concentrations to create a standard curve.
5. Samples measured should be within the concentration range defined by the standard curve, and within the linear dynamic range of the standard curve (Figure 1).
6. If the concentration of an unknown is too high (outside the linear range of the standard curve), dilute the unknown into the linear range and repeat the experiment (Figure 1).
7. Test assay accuracy by measuring controls, i.e. samples where known amounts of purified analyte are spiked into the desired matrix. The recovery values should fall within an acceptable range (± 20%).
8. Test assay precision by measuring replicates of the same sample and calculating the coefficient of variance (CV). Two or three replicates can be used depending on the assay criteria defined by the user.

Samples and Biosensor Preparation

1. Hydrate biosensors for at least 10 minutes prior to use. Biosensors should be hydrated in a biosensor tray assembly with a hydration plate underneath which has at least 200 µL of hydration buffer at corresponding locations. The buffer used for hydration should be identical to the buffer of the samples to be measured.
2. Hydration buffer should ideally be fresh for every biosensor that needs to be hydrated. Do not reuse the hydration buffer to hydrate additional biosensors.
3. Once biosensors are wet, they must stay wet and should not be allowed to dry. They may be stored at 4°C, with the tips of the biosensors submerged in hydration buffer for up to one day, with the understanding that the storage time is determined by the stability of the proteins on biosensors which should be validated by the user. If the biosensors dry out, they lose activity and cannot be used any more.
4. Always prepare all samples including dilutions before starting a Octet® N1 measurement. Use gentle tapping or pipetting up and down to mix samples. Do not vortex samples to avoid formation of bubbles. If bubbles form, perform a quick spin to remove them.

Running the Assay

1. The drop holder should be cleaned thoroughly and dried completely before use. Always clean the drop holder immediately after use, do not let it dry before cleaning. For details on the cleaning procedure, please refer to the Getting Started section, step 6.
2. During measurement, follow the on-screen prompts to enter assay settings (experiment settings, run settings, and sample ID), open the cover, pipette samples into the drop holder, mount the biosensor, and close the cover as instructed. Always pipette samples into the drop holder before mounting a biosensor. The time between mounting a biosensor to measurement should be as short as possible, ideally within 15 seconds.

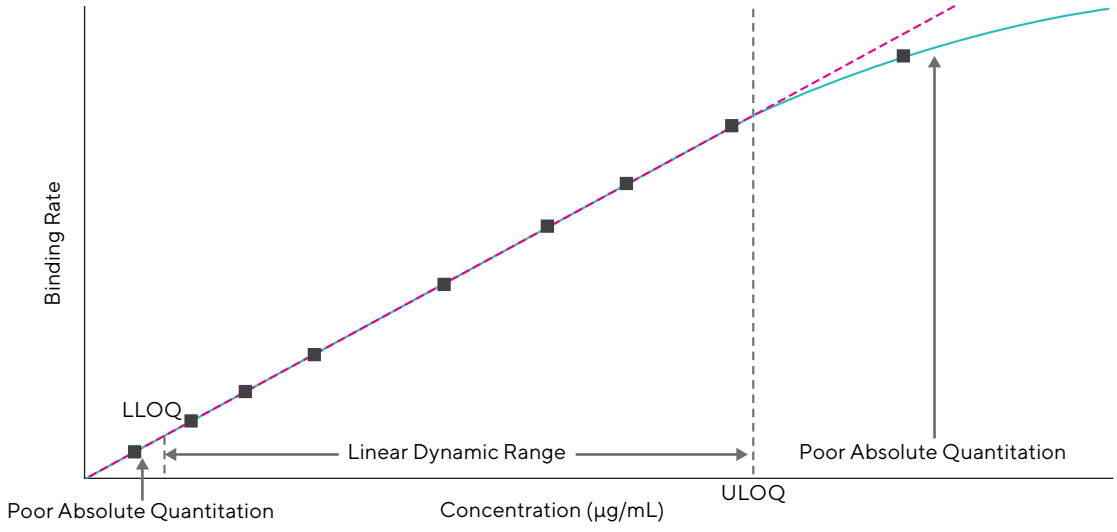

Figure 1: Standard curve showing limits of detection and quantitation. For optimal results, always work within the linear dynamic range of the standard curve.

3. Use the Create Standard Curve function to create standard curves and the Quantitate Sample function to quantitate unknowns.
4. The drop holder should be used for quantitation assays. Pipette 4 µL of sample into the drop holder. Avoid introducing air bubbles when pipetting.
5. Multiple drop holders can be used in a staggered fashion to improve workflow.
6. Once used, a biosensor should be discarded or regenerated. A spent biosensor cannot be reused without proper regeneration.
7. When saving standard curves to reuse for other unknowns at a later time, always note the standard name, matrix, specific ambient temperature at the time, shaking speed, and date to compare curve data across multiple days or weeks.
8. Only load a standard curve that has matched matrix, matched protein, matched shake speed, and matched temperature for calculating the concentration of unknowns.
9. A new standard curve should be created if the lamp has been changed and calibrated. Samples measured after a lamp change should use this new standard curve to calculate concentrations.

Data Analysis

During data analysis:

- Define the biosensor with the buffer blank measurement as a reference by checking the **Ref.** box.
- Subtract the reference biosensor.
- For standard curve application, always first apply the linear-point-to-point function as a curve fitting function and observe the binding trend of the data. Then apply 5PL or linear fits and look for the residual of the curve fitting. If 5PL or linear fits do not perform well, use the linear-point-to-point curve function. Exclude outlier data points if they skew the curve fitting.
- Ideally, only include data points with calculated binding rate values less than 2. This is a user-defined value, but our general recommendation is 2 or less.
- Inspect the equation, equation parameters, R^2 and Chi^2 of the standard curve. Choose a standard curve with R^2 greater than 0.95 and Chi^2 less than 3.
- Inspect the locations of unknown samples on the standard curve. If they are outside the concentration range defined by the standards, the results of these unknowns are not reliable and should be excluded. If the unknowns are located on the upper end of the standard curve that is plateauing out of the curvature (outside the linear dynamic range), the results are not ideal for accurate quantitation. Sample measurement should be repeated by diluting the samples so the concentrations fall within the linear dynamic range of the standard curve (Figure 1).
- Copy and paste data from the table into an Excel file as needed for any additional calculations.
- Click on **Create Report** to save report as a PDF file.

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