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Protein A Biosensors

Octet Biosensors for Label-Free Detection of protein binding
Product Code: 18-5010, 18-5012, 18-5013

Read the entire product insert fully before beginning the assay.

OVERVIEW

Protein A is a cell wall protein derived from *Staphylococcus aureus* that exhibits unique binding properties for immunoglobulins in a variety of mammalian species. In particular, Protein A binds with the Fc region of IgG. A variety of methods for purification and analysis based on Protein A are widely used both in the development and production of antibody and protein therapeutics.

INTENDED USE

ForteBio Protein A Biosensors, in conjunction with the Octet System, are designed for monitoring antibody concentrations in crude lysates, cell culture supernatants and other media. Using Protein A Biosensors, the Octet System supports applications from cell culture screening to purification monitoring during the process development and production of biotherapeutics.

PRINCIPLE

The Protein A Assay for determining protein concentration is based on the rate of binding of a protein of interest to the sensor surface. Different protein concentrations result in different binding rates. The Octet System software calculates the binding rates from standards with known values to generate a standard curve. The binding rate is proportional to the standard concentration. Concentrations of experimental samples are calculated based on their binding rate from the standard curve.

KIT CONTENTS

Protein A Biosensors for the Octet contains:

| Product Ordering Code | | 18-5010 | 18-5012 | 18-5013 |
|-----------------------|---|----------|-----------|------------|
| 1 | Tray of 96 biosensors coated with Protein A | 1x96 | 5x96 | 20x96 |
| 2 | Sample Diluent, 50 mL | 1 bottle | 5 bottles | 20 bottles |

Store Protein A Biosensors in a dry place at room temperature away from direct sunlight. Dispose of Protein A Biosensors as sharps. Upon receipt, Protein A Biosensors are stable for 6 months.

Sample Diluent - (pH 7.4) contains 0.02 % Tween 20, 150 mM NaCl, 1mg/mL BSA in 10 mM Phosphate Buffered Saline (PBS) Solution, 0.05% Sodium Azide. Store @ 2-8°C until expiration.

ADDITIONAL MATERIALS REQUIRED

The following additional materials are required:

- Two (2) 96-well, black, round, flat bottom, polypropylene microplates (Greiner Bio-one # 655209)
- 10x protein stock standard and appropriate diluent e.g. blank media
- Octet Instrument and Software version 3.0 or higher

TECHNIQUES FOR OPTIMAL PERFORMANCE

1. Equilibrate reagents and samples to room temperature prior to preparation. For frozen samples, thaw and mix thoroughly prior to use.
2. Hydration (pre-wet) of the sensors is required prior to assaying on the Octet.
3. A minimum of 200 µL/well is required for both the unknown samples and the sensor hydration solution.
4. Ensure that the Octet instrument is turned on and the lamp is warmed up to room temperature for at least 40 minutes prior to starting the assay.
5. Set the sample plate temperature. In the Octet Software select: **File → Experiment → Set plate temperature**. Enter the desired temperature between room temperature to 40°C. ForteBio recommends assaying at 30°C.

PROTOCOL OVERVIEW

1. Prepare standards and samples and transfer 200 µL of each into a sample plate.
2. Transfer 200 µL of an appropriate hydration solution, which matches the sample and standard matrix, to the 96-well plate. Insert the hydration plate, followed by the sensors onto the sensor tray to hydrate the sensors.
3. Equilibrate both the hydrated sensor assembly and sample plate for 10 minutes on the Octet.
4. Set up the experiment using Octet software.
5. Start the assay.
6. Perform data analysis and save the results.

STANDARD AND SAMPLE PREPARATION

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

Protein A Biosensor dynamic range -

A dynamic range of 2-3 logs is typical. The actual range will be protein dependent. The Protein A Biosensor has been shown to quantitate in the range of 1-500 µg/mL for human polyclonal IgG.

Note: For more accurate quantitation of your protein using Protein A Biosensors on the Octet System, it is recommended to use a purified protein that is identical to the protein of interest.

1. **Standard preparation -** A 10x stock of the purified protein is recommended as the starting material for the standard curve. A total of 200 µL/well will be needed for each test point.

Prepare standards using a diluent similar to the matrix of the unknown samples, *e.g. blank media or the sample diluent supplied*. Prepare a standard series covering the dynamic range for your protein.

- 2 logs - 8 standards
- 3 logs - 12 standards

For some applications, such as screening cell-culture samples, a protein standard is unavailable. It is recommended to use an isotype-matched antibody for relative quantitation of unknown samples.

2. **Unknown samples -** Samples within the established dynamic range can be assayed neat; however certain applications may require dilution.

The table below outlines sample handling for common sample types.

| Sample Type | Handling |
|----------------------|--|
| With-in assay range | Assay neat. Check linearity of dilution across the range to assess any matrix affects. |
| Out of range samples | Dilute out of range samples using the same matrix that matches the standards and unknowns, <i>e.g. blank media (preferred) or sample diluent</i> . Start with a 1:5 dilution factor. |
| New samples types | Include a negative control to test for non-specific binding. |
| Bioreactor samples | Applications using bioreactors that are supplemented may require some modifications to the protocol. Contact support@fortebio.com . |
| Low volume samples | Applications producing low volume samples should be assayed by diluting all samples with a single dilution factor that brings them within the established assay range. |

SENSOR AND SAMPLE PLATE PREPARATION

The following is an example of a sample plate map. It is recommended to setup standards and unknown samples in a column format, which is consistent with sensor sampling on the Octet System.

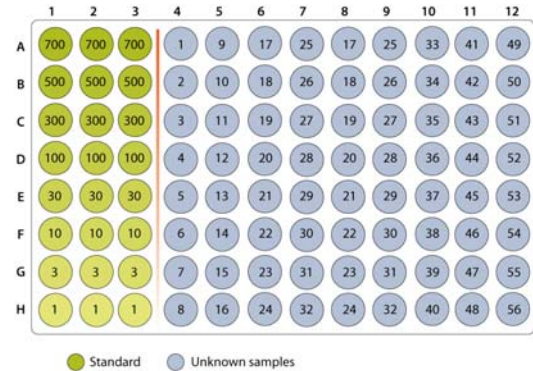


Figure 1. Sample plate diagram for the Octet Protein A Assay

It is critical that the sensors are hydrated with an appropriate solution. ForteBio recommends matching the sensor hydration solution to the standard and sample matrix, *e.g. blank media or sample diluent*.

1. Sample plate preparation -

Transfer 200 µL of each standard and/or unknown into the 96-well black, flat bottom, polypropylene microplate. Gently tap the plate on the bench to remove any bubbles.

2. Sensor hydration plate preparation -

Gently remove the yellow sensor rack from the sensor assembly and place on the bench. Place a 96-well plate securely in the blue sensor tray holder. Transfer 200 µL of the hydration solution into each well in the microplate that matches the number and location of the sensors being used. The table below describes recommended hydration solutions based on experimental sample conditions.

| Experimental Sample Conditions | Standard Diluent | Hydration Solution |
|--|------------------|--------------------|
| Sampled neat in media | media | media |
| Samples requiring dilution - Diluted with media (at least 1:5) | media | media |
| Samples requiring dilution - Diluted with Sample Diluent (at least 1:5) | Sample Diluent | Sample Diluent |

3. Instrument placement of sensors and sample plate


Place the sample plate with A1 facing the upper right hand corner of the sample plate stage on the right hand side of the Octet instrument.

It is critical to hydrate the Protein A Biosensors for 10 minutes on the Octet System. Prior to running, replace the sensor rack by aligning it over the hydration plate, careful not to scrape or touch the bottom of the sensors. Place the sensor tray on the sensor plate stage on the left hand side of the Octet instrument.

Ensure that both the sensor tray and sample plate are securely in place.

INSTRUMENT PREPARATION AND PROGRAMMING

Ensure that the Octet instrument and its computer are turned on. It is essential that the lamp is warmed up for at least 40 minutes.

Double click on the Octet User Software icon  and allow the Octet to complete initialization. From the menu, select **Experiment** → **New Quantitation Experiment** (Ctrl+Q). Fill in the required information for each tab in the following order:


1 Sensor <-> Sample Assignment

1. Use the mouse to highlight the sensors (squares) being used in the sensor plate.
2. Double click on the sample wells (circles) to assign sensors to samples or match the sensor plate. Sensors and the corresponding samples are color matched.

2 Sample Plate Definition

1. Select a Protein A Assay from the Assay listing
2. For unknown samples, enter the dilution factor (if applicable). Enter "1" for undiluted samples. Only 1 dilution factor can be entered for all samples.
3. Identify sample types as either a standard, unknown or control by selecting the wells and right clicking the mouse to select the appropriate group.
 - 3.1. For serial dilution of standards, the Octet System can automatically calculate the standards values. Select all standards wells and right click to select "Set well data". Select dilution and enter the appropriate information for your standard dilutions series.
4. Additional sample information can be entered directly into the table.
5. Save the method file for future use by going to **File** → **Save Method File**. This will save all programming to this point as a method file with the extension ".fmf".
 - 5.1. To recall a previously saved method, go to **File** → **Open** → **Open Experiment Method** (Ctrl+O). After recalling a method file, select the desired Protein A Assay under the Assay listing.


3 Run Experiment

1. Select the browse button to specify a location to save the raw data. It is strongly recommended to save the data to the local drive(C:/). Once the run is complete, the data can then be transferred to a network drive.
2. Enter a unique Experiment Run Name (sub-directory), which creates a new folder for all raw data files and real-time binding charts related to the experiment. Enter a plate name if default name is not acceptable.
3. Default settings will ensure the runtime charts will be saved automatically and data analysis will start at the end of the run. If the sensor and sample plate have not equilibrated on the instrument for 10 minutes, set the delay to start time (seconds).
4. Start the assay by clicking .

DATA ANALYSIS

The Octet has the capability to automatically analyze the data after completion of the run or analyze data offline.


Automated data analysis -

1. Octet will automatically plot the standard curve if standards are present from the previous section.
 - 1.1. To load a previously saved standard curve, select **Data Analysis** → **Load Quantitation Standard Curve** and select a saved standard curve with a file extension "filename.fsc"
2. Under the Standard Curve box, select **Linear Point to Point** curve fitting from the pull down menu.
3. From the menu, select **Data Analysis** → **Recalculate**
4. To calculate unknown results click .


Offline data analysis -

From the menu, select **Data Analysis** → **New Quantitation Data Analysis** (Ctrl_Shift+Q). Fill in the required information for each tab in the following order:

1 Data File Selection

1. Locate and load your raw data files (filename.frd) by double clicking on the folder or individual sensor files.
 - 1.1. To easily locate quantitation files, select the "Show Explorer" button at the bottom of the window. Quantitation folders are colored light purple. Individual ".frd" files are marked as follows: 
 - 1.2. To remove files, simply click the check box under file #.

2 Standard Curve

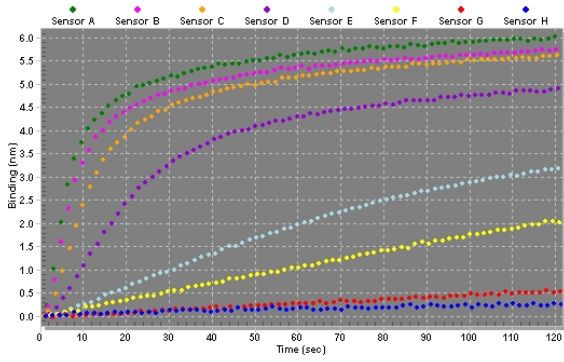
5. Octet will automatically plot the standard curve if standards are present from the previous section.
 - 5.1. To load a previously saved standard curve, select **Data Analysis** → **Load Quantitation Standard Curve** and select a saved standard curve with a file extension "filename.fsc"
6. Under the Standard Curve box, select **Linear Point to Point** curve fitting from the pull down menu.
7. From the menu, select **Data Analysis** → **Recalculate**
8. To calculate unknown results click .

3 Concentration Results

1. Octet will automatically provide the following:
 - 1.1. Unknowns plotted on the standard curve graph
 - 1.2. Graphical plate map of unknown values
 - 1.3. Table of unknown calculated values
2. To save the results, select **File** → **Save Quantitation Data Analysis Report**. A comprehensive word report will open containing all graphs, tables, data, parameters and associated file names.

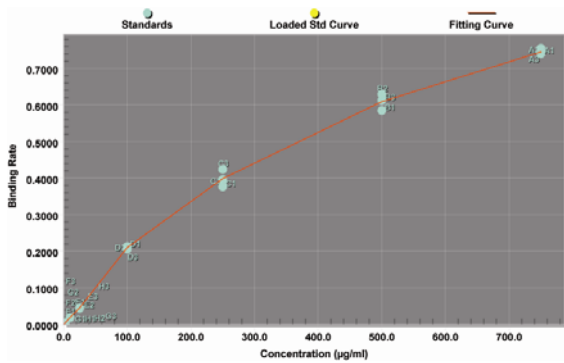
REPRESENTATIVE DATA

Example of an Octet System real-time binding chart using Protein A Biosensors.

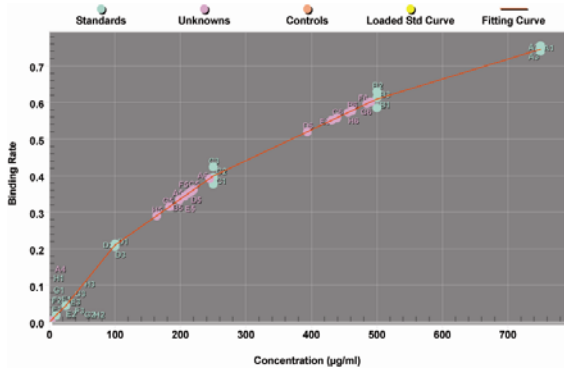


A typical standard curve and unknowns samples are illustrated in the graphs below using linear point to point curve analysis.

Standard Curve



Unknown Concentrations plotted on the standard curve



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