



For additional information on BLI Technology or Technical Support, contact ForteBio or visit the website.

Corporate Headquarters

Tel: 650-322-1360 or 888-Octet-QK
 Fax: 650-322-1370
 1360 Willow Road, Suite 201
 Menlo Park, CA 94025 USA
 Email: info@fortebio.com
 Web: www.fortebio.com

ForteBio name and logo are trademarks of ForteBio, Inc.
 ForteBio™ Copyrights 2007

Anti-Murine IgG Biosensors

Octet Biosensors for Label-Free Detection of protein binding
Product Code: 18-5022, 18-5023, 18-5024

Read the entire product insert fully before beginning the assay.
 For research use only, not for use in diagnostic procedures.

OVERVIEW

The Anti-Murine IgG Biosensor is functionalized with a goat anti-mouse IgG antibody specific for the F(ab')₂ fragment. This antibody recognizes all subtypes of mouse and rat IgG and light chains of other mouse and rat immunoglobins. The antibody is shown to have minimal cross reactivity with human, bovine and horse, rabbit immunoglobins.

INTENDED USE

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

PRINCIPLE

The Anti-Murine IgG Assay for determining IgG concentration is based on the rate of binding of the IgG of interest to the biosensor surface. Different IgG concentrations result in different binding rates. The Octet System software calculates the binding rates from standards with known concentrations 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

Anti-Murine IgG Biosensors for the Octet System contains:

Product Ordering Code		18-5022	18-5023	18-5024
1	Tray of 96 biosensors coated with anti-mIgG F(ab') ₂	1x96	5x96	20x96
2	Sample Diluent, 50 mL	1 bottle	5 bottles	20 bottles

Store Biosensors in a dry place at room temperature away from direct sunlight. Dispose of Biosensors as sharps. Upon receipt, 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 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)
- IgG standard (>1mg/mL stock preferred) and appropriate diluent e.g. blank media. For best performance the standard should be of the same species and subtype as the unknown samples.
- Octet Instrument and Software version 3.2 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 biosensors is required prior to assaying on the Octet.
3. A minimum of 200 µL/well is required for both the unknown samples and the biosensor 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 biosensors onto the Octet instrument to hydrate the biosensors.
3. Equilibrate both the hydrated biosensor assembly and sample plate for 10 minutes on the Octet instrument.
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.

Anti-Murine IgG Biosensor dynamic range -

A dynamic range of 2 logs is typical. The actual range will be protein dependent. The Anti-Murine IgG Biosensor has been shown to quantitate in the range of 1-100 $\mu\text{g}/\text{mL}$ for mouse polyclonal IgG.

Note: For more accurate quantitation of your protein using anti-Murine IgG 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 (over the highest standard concentration) of the purified protein is recommended.
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. For the typical 2 log range of the assay, it is recommended to have 8 standards covering this range. A total of 200 μL per well will be needed for each point.

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

2. **Unknown samples**- The Anti-Murine IgG Biosensors recognize all subtypes of mouse and rat IgG. The biosensors will also recognize and quantitate the light chains of other immunoglobins present.

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.</i> blank media (preferred) or sample diluent. 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 to bring them to a 200 μL volume.

BIOSENSOR 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 biosensor sampling on the Octet System.

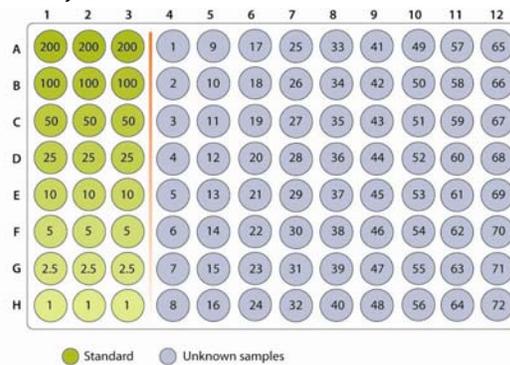


Figure 1: Sample plate diagram using Anti-Murine IgG Biosensors

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. **Biosensor hydration plate preparation** - Gently remove the upper biosensor rack from the biosensor assembly and place on the bench. Place a 96-well plate securely in the blue biosensor tray holder. Transfer 200 μL of the hydration solution into each well in the microplate that matches the number and location of the biosensors being used.

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

3. **Instrument placement of biosensors 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 biosensors for 10 minutes on the Octet System. Prior to running, replace the biosensor rack by aligning it over the hydration plate, careful not to scrape or touch the bottom of the biosensors. Place the biosensor tray on the biosensor plate stage on the left hand side of the Octet instrument.

Ensure that both the biosensor 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 biosensors (squares) being used in the biosensor plate.
2. Double click on the sample wells (circles) to assign biosensors to samples or match the biosensor plate. Biosensors and the corresponding samples are color matched.

2 Sample Plate Definition

1. Select Anti-Human IgG Standard Assay from the Assay Settings list. The Anti-Murine IgG assay parameters are identical to those set for the standard Anti-human IgG assay.

Special note: For rat IgG species, select Custom Quantitation. The assay parameters are slightly different and need to be modified. Contact support@fortebio.com for assistance.

2. Identify sample types as either a standard, unknown or control by selecting the wells and right clicking the mouse to select the appropriate group.
 - 2.1. 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.
 - 2.2. If applicable, enter dilution factors for each sample in the sample plate table. A default dilution factor of 1 is assumed if none is entered.
 - 2.3. Additional sample information can be entered directly into the table.
3. Save the method file for future use by going to File → Save Method File. The Method will automatically be saved once the assay is started. The method file is saved with the extension of “filename.fmf”.
 - 3.1. To recall a previously saved method, go to File → Open → Open Experiment Method (Ctrl+O).

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 biosensor 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 Dose Response-5PL curve fitting from the pull down menu.
3. From the menu, select Data Analysis → Recalculate
4. To calculate unknown results click .

3 Concentration Results

1. The 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 report can be saved as either a Microsoft Word® or Microsoft Excel® document.

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 biosensor 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

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 Dose Response-5PL curve fitting from the pull down menu.
3. From the menu, select Data Analysis → Recalculate
4. 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 report can be saved as either a Microsoft Word® or Microsoft Excel® document.

REPRESENTATIVE DATA

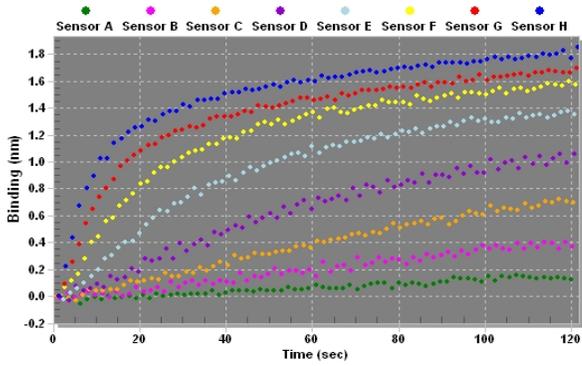


Figure 2. Typical real-time binding chart using Anti-Murine IgG Biosensors to assay mouse IgG standards at 200, 100, 50, 25, 10, 5, 2.5, and 1 µg/mL.

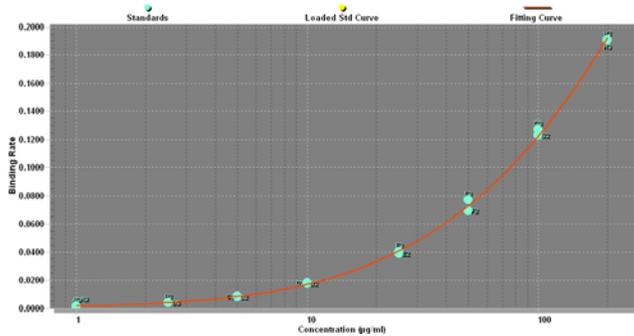


Figure 3. A typical standard curve from mouse IgG standards using a Dose Response-5PL fit.

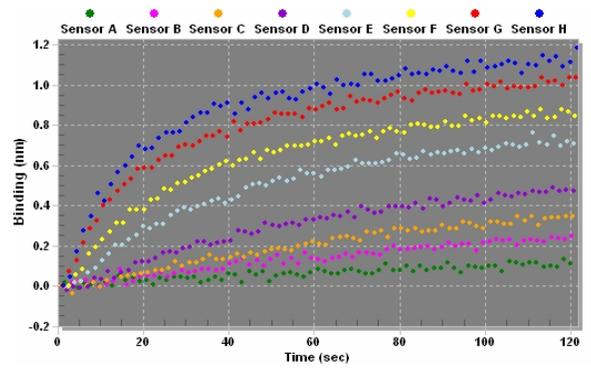


Figure 4. An example of a real-time binding chart using Anti-Murine IgG Biosensors bind to rat IgG standards at 200, 100, 50, 25, 10, 5, 2.5, and 1 µg/mL.

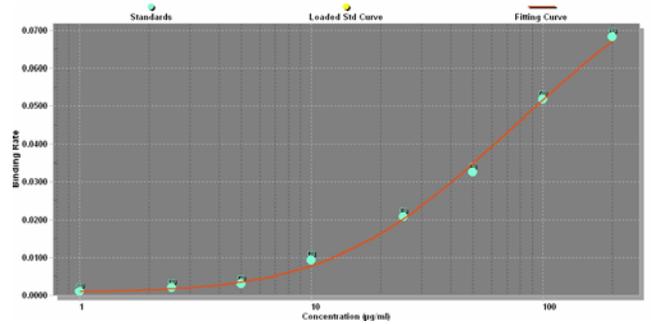


Figure 5. An example standard curve from rat IgG standards using a Dose Response-5PL fit.

**Technical Support: Toll Free (888) OCTET-QK
Phone (650) 322-1360 Option 3**