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Streptavidin High Binding Capacity Biosensors
-Kinetics and Screening Grade

Octet Biosensors for Label-Free Detection of protein binding
Product Codes: 18-5019, 18-5020, 18-5021 (Screening Grade)
18-5042, 18-5043, 18-5044 (Kinetics Grade)

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

OVERVIEW

The Streptavidin High Binding Biosensors enable the immobilization of biotinylated proteins onto the biosensor surface. The immobilized protein can then be used in subsequent kinetic interaction analysis. The biotin-streptavidin interaction is an extremely stable interaction which makes it suitable for demanding kinetics applications.

INTENDED USE

ForteBio Streptavidin High Binding Biosensors, in conjunction with the Octet System, are designed for kinetic analysis of macromolecular interactions. The Streptavidin High Binding Biosensor is intended for a variety of kinetics applications. The *Screening Grade* Streptavidin High Binding Capacity Biosensors are optimal for applications that require only 15 minutes of baseline stability while the *Kinetics Grade* Streptavidin High Binding Capacity Biosensors are intended for use in application where 60 minutes of baseline stability is desired. The *Screening Grade* Biosensors are also ideal for the development of custom quantitation assays for a biotinylated protein of interest. The streptavidin biosensors can be used with many sample types including purified proteins, cell culture supernatants, and cell lysates.

PRINCIPLE

Streptavidin is a 60Kd tetrameric protein isolated from *Streptomyces avidinii* and has an extremely high affinity for the small molecule biotin (K_D reported at 10^{-15}M). The Streptavidin Biosensor surface has a biocompatible layer on which streptavidin has been immobilized. This surface allows for the quick and stable immobilization of any biotinylated protein, peptide, dsDNA or oligo. Best results are seen with biomolecules having a low molar coupling ratio of biotin to protein and using biotinylation reagents which incorporate a linker to allow for greater protein mobility once immobilized. Once the biotinylated molecule is immobilized onto the biosensor the resulting stable surface is suitable for most kinetic applications.

KIT CONTENTS

Streptavidin High Binding Biosensor Kit for the Octet System contains:

Product Ordering Code and sizes		1 tray	5 trays	20 trays
1	Streptavidin High Binding Capacity Biosensors (Kinetics Grade) (tray of 32 sensors)	**18-5042**	**18-5043**	**18-5044**
1	Streptavidin High Binding Capacity FA Biosensors (Screening Grade) (tray of 96 sensors)	**18-5019**	**18-5020**	**18-5021**
-	10X Kinetics Buffer, 50 mL (included in kit)	1 bottle	1 bottles	4 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.

10X Kinetics Buffer - 10mM Phosphate, 150 mM NaCl, 0.02% Tween 20, 0.05% Sodium Azide, 1 mg/mL BSA (pH 7.4). Store @ 2-8°C until expiration. **Dilution to 1X should be made with PBS prior to use.**

ADDITIONAL MATERIALS REQUIRED

The following additional materials are required:

- **Biotinylated-ligand -** Biotinylation at a low biotin:protein ratio with longer linker reagents such as Biotin-LCLC-NHS (Pierce # 21338) or Biotin-PEO4-NHS (Pierce # 21329) typically gives the best results. Please refer to the Technical Note TN3006 on Biotinylation for a complete protocol.
- **Interacting protein(s)**
- **Assay Buffer -** 1X Kinetics Buffer recommended. Dilute 10X Kinetics Buffer stock with PBS. Other buffers may be used. Best results seen when the buffer is kept consistent throughout hydration and all assay steps
- **Octet Instrument and Software** version 3.0 or higher
- 96 well Microplates - 2 X 96-well, black, flat bottom, polypropylene microplates (Greiner Bio-one # 655209)

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 of the sensors is required prior to assaying on the Octet System. Hydration of the biosensors in a buffer consistent with the buffer used throughout the assay will give the most stable results.

3. A minimum volume of 200 µL/well is required for both the assay 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 by selecting **File ➔ Experiment ➔ Set plate temperature**. Enter the desired temperature. ForteBio recommends assaying at 30°C. Assaying at other temperatures may require different assay times than discussed in this protocol.

PROTOCOL OVERVIEW

1. Prepare buffers, biotinylated- ligand and interacting protein samples.
2. Transfer 200 µL of an appropriate hydration solution to the 96-well plate. Insert the hydration plate, followed by the biosensors onto the biosensor tray to hydrate the sensors.
3. Transfer 200 µL of each appropriate sample or buffer into the appropriate wells of a 96-well plate.
4. Equilibrate both the hydrated biosensor assembly and sample plate for **15 minutes** on the Octet instrument.
5. Start the assay.
6. Perform data analysis and save the results.

SAMPLE PREPARATION

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

1. Buffer – Prepare buffer. It is recommended to use 1X Kinetics Buffer. To prepare 1X buffer, dilute the 10X buffer with PBS at a ratio of 1 part 10X Kinetics Buffer to 9 parts PBS. Use this buffer for biosensor hydration, all reagent dilutions, baseline steps, and dissociation steps.

2. Biotinylated-ligand – The biotinylated-ligand is the protein that is immobilized onto the streptavidin biosensor tip surface. Biotinylation at a low biotin:protein ratio is recommended. Biotinylation reagents with a longer linker such as Biotin-LCLC-NHS (Pierce # 21338) or Biotin-PEO4-NHS (Pierce # 21329) typically give the best results. Please refer to the Technical Note TN3006 on Biotinylation for the recommended protocol.

For the most reproducible surface capacity, loading at a high concentration of biotin-ligand (50 µg/mL) and a low flow rate (0 rpm) is recommended. If the biotin ligand amount is insufficient to reach this concentration, loading at a lower concentration (25-50 µg/mL) under higher flow (800-1000rpm) typically gives good precision as well. The biotinylated ligand reagent should be made in 1X Kinetics Buffer (or the buffer to be used throughout the assay).

A volume of 200 µL of biotin-ligand is needed for each well. The biotinylated-ligand solution can be recovered from the well after the assay and reused if desired.

3. Interacting protein – During kinetic analysis, it is recommended to run at least 4 different concentrations of the interacting protein in the form of a dilution series. The highest concentration should be approximately 10 fold above the expected K_D. For example for an interaction with an expected low nM affinity, concentrations of the interacting protein of 90 nM, 30 nM, 10 nM and 3 nM would typically provide good kinetic data. A well volume of 200 µL of protein solution is required for each sample. For a kinetic screening assay a single concentration of each sample to be tested can be assayed to provide a rapid assessment of kinetics of many samples.

ONLINE IMMOBILIZATION PROTOCOL

For online immobilization using Streptavidin High Binding Biosensors, there are typically 5 Assay Steps that can be fully automated on the Octet System.

1. Baseline (Typically 1X Kinetics Buffer))
2. Loading of biotinylated ligand
3. Baseline (Typically 1X Kinetics Buffer)
4. Association of the interacting protein
5. Dissociation (Typically 1X Kinetics Buffer)

1. Assay preparation- Prepare the biotin-ligand and interacting protein as described in the Sample Preparation section above.

2. Assay Plate - Transfer 200 µL of each assay reagent into the 96-well black, flat bottom, polypropylene microplate according to the plate map in Table 1. Each reagent is transferred to the 96-well microplate in a column format.

	1	2	3	4	5
A	Buffer	Biotin-ligand	Buffer	Interacting protein	Buffer
B	Buffer	Biotin-ligand	Buffer	Interacting protein	Buffer
C	Buffer	Biotin-ligand	Buffer	Interacting protein	Buffer
…	Buffer	Biotin-ligand	Buffer	Interacting protein	Buffer

Table 1. Example of a plate map for online immobilization

3. Biosensor hydration plate preparation - Gently remove the top portion of the 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 sensors being used. It is highly recommended to hydrate the sensors in the same buffer that is used throughout the assay.

Replace the biosensor rack by aligning it over the hydration plate, taking care not to scrape or touch the bottom of the sensors. It is critical to hydrate the Biosensors for at least **15 minutes** on the Octet System.

4. Instrument placement of sensors and sample plate - Place the sample plate with A1 toward the back right corner of upper right hand corner on the sample plate stage in the Octet instrument.

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. Go to **"Instrument preparation and programming".**

INSTRUMENT PREPARATION AND PROGRAMMING

Ensure that the Octet instrument and the 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 Kinetics Experiment** (Ctrl+K). Fill in the required information for each tab in the following order:

Sensor <-> Sample Assignment

1. Use the mouse to highlight the sensors (squares) being used in the biosensor plate. Sensor information can be entered directly into the table.

2. Use the mouse to highlight the wells which contain samples or reagents. Sample information can be entered directly into the table.

3. Enter in the concentration values in for the interacting protein.

Assay Definition

In the Table "**Step data setup**" is an example list of the programmable steps with the associated parameters. The table below lists the order of steps for a typical assay.

Step#	Step name	Time (sec)	Flow (rpm)	Step type
1	Baseline	180-600	0-1000	Baseline
2	Loading	300-600	0-1000	Loading
3	Baseline	180-600	1000	Baseline
4	Protein binding	600-1800	1000	Association
5	Dissociation	600-3600	1000	Dissociation

Table 2. Example assay steps and associated parameters

1. Click on the "+" or "ADD" the number of assay steps needed to perform the assay.
2. Define each assay step.
   - 2.1. Under the "data name", double click to change the name of the step
   - 2.2. Under "Assay Time", enter the time (seconds) for the specific assay step
   - 2.3. Under "Flow Rate", enter the rpm for the orbital flow shaker for each step.
     - **2.3.1. Best results are typically seen by loading at high biotin-ligand concentration (~50 µg/mL) at 0 rpm.**
     - 2.3.2. If loading must be done at a lower concentration, then a flow rate of 800-1000 rpm is recommended.
     - 2.3.3. **All kinetics steps (Baseline, Association and Dissociation steps) should be performed at a flow rate of 1000 rpm.**
   - 2.4. Under "Type", select the step type from the pull down menu.
3. Create the assay protocol by assigning the biosensors, samples and their associated assay steps in the order they should be performed.
   - 3.1. Select a column of biosensors by clicking on the column that contains the biosensors you wish to use.
   - 3.2. Select the first defined assay step from the **Step data setup** table by clicking on it.
   - 3.3. Double-click on the sample column to be used in the selected step. The step will appear in the "Assay Setup List"
   - 3.4. Complete this for the remaining assay steps prior to selecting the next column of biosensors.

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 the default name is not acceptable.
3. Check the "Delay the experiment start box". Enter 900 seconds to allow for the 15 minutes of equilibration time needed for the samples to come to the assay temperature.
4. Default settings will ensure the runtime charts will be saved automatically.
5. Start the assay by clicking .

DATA ANALYSIS

From the menu, select **Data Analysis ➔ New Kinetics Data Analysis** (Ctrl+Shift+K). Fill in the required information for each tab in the following order:

Data File Selection

Locate and load your raw data files (filename.frd) by double clicking on the folder or individual biosensor files.

1. To easily locate kinetic files, select the "Show Explorer" button at the bottom of the window. Kinetic folders are colored peach .
2. To remove files, simply click the check box under file #.

Data Visualization

In the Data Visualization screen, the raw data for all sensors assayed are presented in graphical form. This section allows for customization of how the data is graphically presented and for setting the parameters to be used in kinetic data analysis.

1. A thumbnail graphic of each individual biosensor is presented on the left had side of the screen. Select or deselect the check box the biosensors for analysis
2. For better visualization, the data can be aligned by assay step type.
   - 2.1. Select the step type to align to by using the pull down menu
   - 2.2. Select which steps you would like presented
   - 2.3. Select which axis to align
   - 2.4. The image and the raw data can be exported as a jpeg and text file, respectively.
3. Using the thumbnails on the left side of the window, a reference sensor can be designated and subtracted from all other sensor data shown.
4. Under **Data Tracking**, select how the biosensors should be identified in data tables and graphs.
   a. Sensor Loc – biosensor location, e.g. B1
   b. Sample loc – sample location
   c. Sample ID
5. Under **Assay Step Times,** define the desired time frame for analysis. Octet System software gives the option of selecting either the time that is stored in the method file or overriding the programmed time for either association or dissociation.

6. If desired, the entire displayed data set may be analyzed using Global Fitting to calculate a single k_a, k_d and K_D, by checking the box next to Global analysis. If this box is not checked, each kinetic curve will be analyzed separately.

7. Octet System software for kinetic analysis is based on a 1:1 model. Select full or partial dissociation based on the experiment and understanding of the interaction

8. To calculate kinetic constants click  .

FORTEBIO KINETIC ANALYSIS

The Octet System software has standard kinetic analysis and reporting based on the selected data established in the Data Visualization section.

1. In order to calculate affinity constants, a molar concentration is required. If the concentration of the interacting protein was not entered during the assay set-up, enter the concentration in molar units at the top of the spreadsheet. *e.g. 4 E-8 is equivalent to 40 nM*
2. In the "ForteBio data analysis fitting window": Select the Data Analysis settings for inclusion in the Microsoft® Word report. The available options are listed in the Table 3.
3. Select the settings for inclusion in the report.

#	Data Analysis Setting	Description
1	Create stacked graph	For each biosensor selected, displays individual on/off binding data and associated residuals
2	Create one overlay graph	Displays binding data and residuals in a single graph
3	Draw fitted curve on the graph	Displays the non-linear fitted curve for each biosensor
4	Show curve legend	Displays a legend
5	Show residual curve	Displays the difference between the actual binding rate and the curve fit
6	Create results chart	A table of all calculated data for each biosensor.

Table 3. Available kinetic data analysis settings for reporting

4. Click on the "**Do Analysis"** button.
5. After the analysis is complete, you can save the report and are given the following options:
   a. Tables and graphs
   b. Graphs only
   c. Tables only
6. Choose the options to include in the report
7. Click the "**Save Report As"** button to save a Microsoft® Word document report.

Note: For more advanced kinetics analysis the data can be analyzed in Origin®. Refer to the Origin manual for more information.

REPRESENTATIVE DATA

Figure 1: *Example of an Octet System real-time binding chart using Streptavidin HBC Biosensors. Data shown is for the immobilization of biotin anti-TNFα antibody followed by the kinetic analysis of TNFα binding at 200, 100, 50, 25, 12.5, 6.25, 3.13 and 0 nM. Antibody purchased from BD Pharmingen (#551220) and biotinylated according to the Technical Note. TNFα protein purchased from eBiosciences (#34-8329-85). Buffer used throughout the assay was the 1X Kinetics Buffer supplied with the biosensors.*

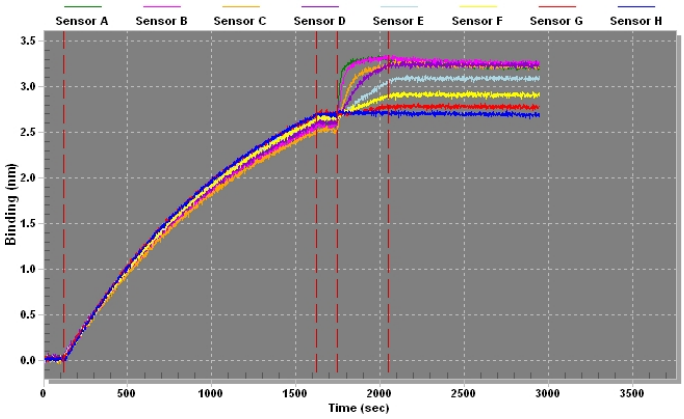
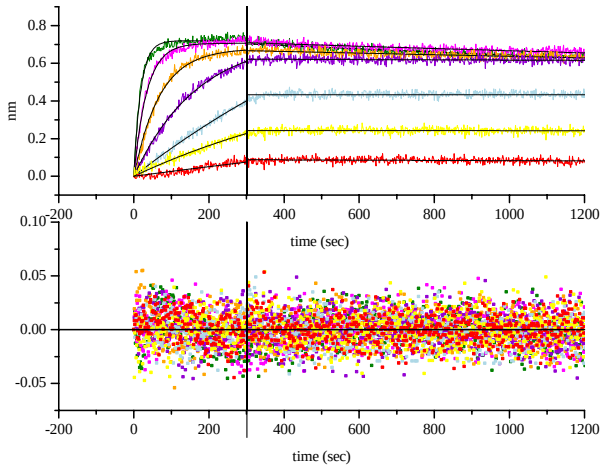


Figure 2: *Data visualization of the model fits with their associated residuals.*



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