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Super Streptavidin Biosensors

Biosensors for Label-Free Detection of Protein Binding on an Octet RED System
Product Codes: 18-5057

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

OVERVIEW

The Super Streptavidin Biosensors enable the immobilization of biotinylated molecules onto the biosensor surface. The immobilized molecule 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 where signal may be limiting.

INTENDED USE

ForteBio Super Streptavidin Biosensors, in conjunction with the Octet RED System, are designed for kinetic analysis of small protein and/or small molecule interactions.

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

The Super Streptavidin Biosensor Kit for Octet RED System contains:

Product Ordering Code and sizes		1 tray
1	Super Streptavidin Biosensors: One tray of 96 biosensors coated with streptavidin for small molecule kinetic analysis.	**18-5057**

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.

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) or small molecules**
- **Assay Buffer** - Best results seen when the buffer is kept consistent throughout hydration and all assay steps. It is recommended to keep DMSO below 10%.
- **Octet RED Instrument and Software** version 5.0 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 RED 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 and a maximum volume of 230 µL/well are required for both the assay samples and the biosensor hydration solution.

4. Ensure that the Octet RED instrument is turned on and the lamp is warmed up to room temperature for at least 60 minutes prior to starting the assay.

5. Set the sample plate temperature in the Octet RED 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 RED 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. Use this buffer for biosensor hydration, all reagent dilutions, baseline steps, and dissociation steps. If using the 10x Kinetics Buffer provided, dilute to 1x with PBS Buffer, prior to use.

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.

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 molecule – During kinetic analysis, it is recommended to run at least 4 different concentrations of the interacting analyte 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 solution is required for each sample.

ONLINE IMMOBILIZATION PROTOCOL

NOTE: For demanding small molecule applications on the Octet RED System a protocol using double referencing is required. Please see the Small Molecule Technical Note for details.

For standard protein kinetics on larger biomolecules assay using Super Streptavidin Biosensors, there are typically 5 Assay Steps that can be fully automated on the Octet RED 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 RED 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 RED instrument.

Place the biosensor tray on the biosensor plate stage on the left hand side of the Octet RED 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 RED instrument and the computer are turned on. It is essential that the lamp is warmed up for at least 60 minutes.

Double click on the Octet User Software icon and allow the Octet RED 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:

1 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.

2 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	600-1800	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.

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 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 GO.

REPRESENTATIVE DATA

Figure 1: *Example of an Octet RED System real-time binding chart using Super Streptavidin Biosensors. Data shown is for the immobilization of biotin anti-His antibody followed by the kinetic analysis of His-tagged Ubiquitin binding at 10, 3.3, 1, 0.33, 0.1 µM. Antibody purchased from Novagen (#70796-3) and biotinylated according to the Technical Note TN3006. His-tagged ubiquitin was purchased from EMD Bioscience (662060). Buffer used throughout the assay was the 1X Kinetics Buffer supplied with the biosensors.*

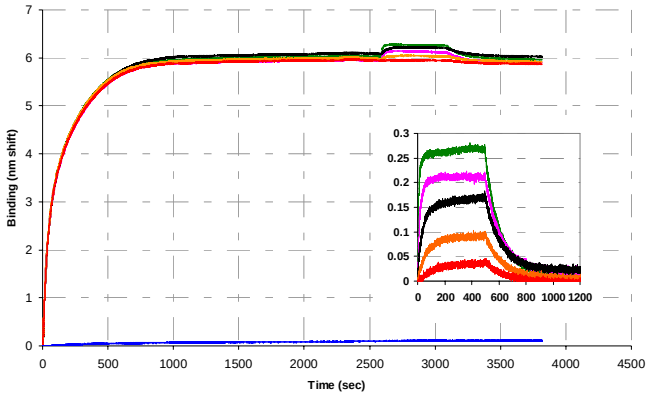
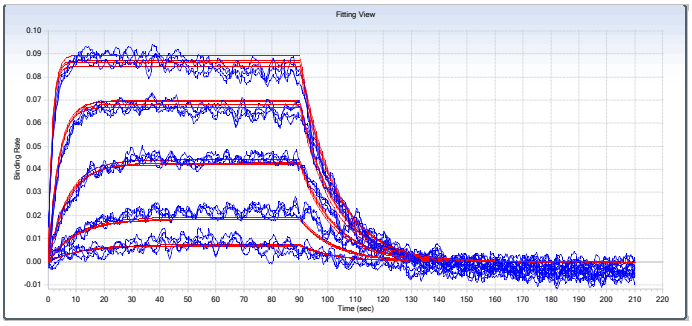


Figure 2: *Data from Octet RED of the kinetic analysis of furosimide (Sigma F4381) binding to immobilized Carbonic Anhydrase (Sigma C2522). Carbonic anhydrase was biotinylated according to the Technical Note TN3006. Furosimide was bound at 10, 3, 1, 0.3, 0.1 µM in PBS with 0.5% DMSO. Five replicates of each concentration are shown. Data was analyzed using Octet Red data analysis software version 5.0. The calculated kinetics constants are shown below.*



k_a (1/Ms)	6.34 E4
k_d (1/s)	8.11E-2
K_D (M)	1.28E-6 ± 6E-8

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