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: support@fortebio.com

Web: www.fortebio.com

Anti-Human IgG Capture (AHC) Biosensors
-Kinetics Grade

Octet Biosensors for Label-Free Detection of Protein Binding
Product Codes: 18-5060, 18-5061

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

OVERVIEW

The Anti-Human IgG Capture (AHC) Biosensors enable the immobilization of human IgG or other proteins containing a human Fc region onto the biosensor surface. The immobilized protein can then be used in subsequent kinetic interaction analysis. The surface of the AHC biosensor has been designed to provide a stable interaction which makes it suitable for demanding kinetics applications.

INTENDED USE

ForteBio Anti-Human IgG Capture (AHC) Biosensors, in conjunction with the Octet QK System or Octet RED System, are designed for kinetic analysis of macromolecular interactions. The AHC Biosensors can be used with many sample types including purified proteins, cell culture supernatants, and cell lysates.

PRINCIPLE

The Anti-Human IgG Capture (AHC) Biosensor is pre-immobilized with a high affinity antibody against the Fc portion of human IgG. This antibody can capture and immobilize human IgG (hIgG) or other Fc containing ligands to produce a stable surface suitable for interaction analysis. The capture surface is particularly suited toward immobilizing hIgG from cell culture supernatants or other complex mixtures where biotinylation is not an option.

The AHC Biosensor has been designed to withstand typical regeneration conditions. In a typical workflow this allows for the capture of the hIgG of interest, the analysis of the human IgG binding to its analyte then the regeneration of the surface back to its original state. The biosensor can then be reused by loading new human IgG.

KIT CONTENTS

Anti-Human IgG Capture Biosensors for the Octet QK and the Octet RED System contain:

Product Ordering Code		**18-5060**	**18-5061**
1a	Anti-Human IgG Capture Biosensor (96 biosensors/tray)	**Tray**	**Quad** *(4 trays)*
1b	10x Kinetics Buffer (50 mL/bottle)	1 bottle	1 bottle

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.

ADDITIONAL MATERIALS REQUIRED

The following additional materials are required:

- **Human IgG -** Human IgG or other human Fc containing proteins can be immobilized. The human IgG can be pure or part of a complex mixture such as culture supernatants.
- **Conditioning/Regeneration Solution –** 10 mM Glycine pH 1.7 is required for surface conditioning and regeneration. Further pH optimization might be necessary depending on the immobilized molecule.
- **Interacting protein(s)**
- **Assay Buffer -** 1X Kinetics Buffer recommended (dilute 10X Kinetics buffer 1:10 with PBS, pH 7.4). Other buffers may be used. Best results seen when the buffer is kept consistent throughout biosensor hydration and all assay steps.
- **Octet QK System or Octet RED System**
- 96-well microplate - 2 X 96-well, black, flat bottom, polypropylene microplates (Greiner Bio-one # 655209)

TECHNIQUES FOR OPTIMAL PERFORMANCE

1. Conditioning of the biosensor is required prior to the first loading step to improve baseline and surface stability. Typically conditioning consists of 3 X 20 second exposures to Regeneration Buffer alternating with 1X Kinetics Buffer.
2. A "prime" of the biosensor surface consisting of a binding and regeneration cycle is recommended for the most rigorous applications. This priming binding cycle should consist of capturing the hIgG from solution and then regenerating back to the original surface using the regeneration protocol. The priming should be done following the conditioning of the surface.
3. Typically regeneration of the biosensor surface can be achieved through 5 X 5 second exposures to 10 mM Glycine pH 1.7 alternating with 1X Kinetics Buffer. In some cases the regeneration may need to be further optimized depending on the hIgG.
4. For optimal performance, a reference biosensor should be included. A reference biosensor should be loaded with the hIgG and should be run with a buffer blank for the association and dissociation steps.
5. Equilibrate reagents and samples to room temperature prior to preparation. For frozen samples, thaw and mix thoroughly prior to use.
6. Hydration of the biosensors 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.
7. A minimum volume of 200 µL/well is required for both the assay samples and the biosensor hydration solution.
8. 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.
9. 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, regeneration solution, 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 biosensors.
3. Transfer 200 uL 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 10 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. **Human IgG or other Fc containing ligand** – The ligand is the protein that is immobilized onto the biosensor tip surface. A typical immobilization concentration for the Fc containing ligand is 5-25 µg/mL. A volume of 200 µL of ligand is needed for each well. The ligand solution can be recovered from the well after the assay and reused if desired.

   If the ligand is to be captured from cell culture supernatants, best results are seen if the supernatant is diluted at least 1:2 with 1X Kinetics Buffer. If this dilution results in a low total concentration of the ligand, the biosensors can be incubated with the cell culture overnight at 4C to maximize loading of the Fc containing ligand. For tips on overnight loading, see the technical note *TN3010_Streptavidin Batch Immobilization*.
2. Interacting protein – During rigorous 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 KD. 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 screening assays or qualitative interaction analysis, a single concentration of the interacting protein can be sufficient to characterize the binding.
3. Conditioning/Regeneration Solution – The Anti-Human IgG Capture Biosensor can be regenerated back to the capture surface level by using 10 mM Glycine pH 1.7. After regeneration the biosensor can be reloaded with hIgG for a new interaction analysis. A small loss in binding capacity has been seen with repeated regenerations. In order to stabilize the surface, an initial conditioning step is recommended. This conditioning consists of exposure of the biosensor to Regeneration Buffer prior to the first interaction assay.

   The pH of the Conditioning/Regeneration solution may need to be further optimized for the specific hIgG/interacting protein analyzed.

ONLINE IMMOBILIZATION PROTOCOL

Below is a list of typical assay steps used in online immobilization. These steps are fully automated by the Octet System.

1. **Baseline** (Typically 1X Kinetics Buffer or media sample)
2. **Conditioning** (recommended). Exposure of the biosensor to the regeneration solution to optimize baseline performance and to stabilize the surface density.
3. **Priming Binding cycle** (optional)
4. **Loading of ligand** (Typically 1X Kinetics Buffer or media sample)
5. **Baseline** (Typically 1X Kinetics Buffer)
6. **Association of the interacting protein** (Typically 1X Kinetics Buffer)
7. **Dissociation** (Typically 1X Kinetics Buffer)
8. **Regeneration** (10 mM Glycine pH 1.7)

1. Assay preparation - Prepare the buffers, regeneration solution, 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	6	7
A	Buffer	10 mM Glycine pH 1.7	Buffer	hIgG or Fc-ligand	Buffer	Interacting protein	Buffer
B	Buffer	10 mM Glycine pH 1.7	Buffer	hIgG or Fc-ligand	Buffer	Interacting protein	Buffer
C	Buffer	10 mM Glycine pH 1.7	Buffer	hIgG or Fc-ligand	Buffer	Interacting protein	Buffer
..	...	...	...	...	...	...	...
H	Buffer	10 mM Glycine pH 1.7	Buffer	hIgG or Fc-ligand	Buffer	Buffer	Buffer

Table 1. Example of a plate map.

3. **Biosensor hydration plate preparation** - Carefully remove the top portion of the yellow biosensor rack from the biosensor tray assembly and place on the bench. Place a black 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 highly

recommended to hydrate the biosensors 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 biosensors. It is critical to hydrate the Biosensors for at least **10 minutes** on the Octet QK System or Octet RED System.

4. **Instrument placement of biosensors and sample plate** - Place the sample plate with A1 toward the back right corner of the sample plate stage in the Octet QK Instrument or Octet RED 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 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 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 Table 2, "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.

If a priming binding cycle is run then the biosensor would be regenerated after the loading of the human IgG or Fc containing ligand (step 9) by using the 5x regeneration cycles. After the priming, the biosensor should be reloaded with the human IgG or Fc containing ligand, and the assay runs from step 8 on.

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. Specify assay time
   - 2.3. Under "Flow Rate", enter the rpm for the orbital flow shaker. **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.

Step #	Step name	Time (sec)	Flow (rpm)	Step type
1	Baseline	180-600	1000	Baseline
2	Conditioning (10 mM Glycine)	20	1000	Custom
3	Buffer	20	1000	Custom
4	Conditioning (10 mM Glycine)	20	1000	Custom
5	Buffer	20	1000	Custom
6	Conditioning (10 mM Glycine)	20	1000	Custom
7	Buffer	20	1000	Custom
8	Baseline	300-600	1000	Baseline
9	Loading	300-600	0-1000	Loading
10	Baseline	180-600	1000	Baseline
11	Protein binding	600-1800	1000	Association
12	Dissociation	600-3600	1000	Dissociation
13	Regeneration (10 mM Glycine)	5	1000	Custom or Regen*
14	Buffer	5	1000	Custom
15	Regeneration (10 mM Glycine)	5	1000	Custom or Regen*
16	Buffer	5	1000	Custom
17	Regeneration (10 mM Glycine)	5	1000	Custom or Regen*
18	Buffer	5	1000	Custom
19	Regeneration (10 mM Glycine)	5	1000	Custom or Regen*
20	Buffer	5	1000	Custom
21	Regeneration (10 mM Glycine)	5	1000	Custom or Regen*
22	Buffer	5	1000	Custom

Table 2. Example assay steps and associated parameters. After regeneration, steps 8-22 would be repeated to reload and perform the next assay. * On Octet QK use Regen step type. On Octet RED use Custom step type.

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. Check the "Delay the experiment start box". Enter 300 seconds to allow for the 5 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:

1. Locate and load data as outlined in the instrument and software manual.
2. The reference biosensor (a biosensor on which the hIgG or Fc containing ligand was loaded but no interacting proteins was assayed) should be used for reference subtraction.
3. Complete the data analysis according to the instrument and software manual.

REPRESENTATIVE DATA

Figure 1a: *Example Run data. Assay shown in real time binding chart consists of 5 binding cycles separated by regeneration of the surface. Surface conditioning proceeded the first binding cycle. Biosensors A-C show a titration series of the Analyte and Biosensor D was used for reference subtraction.*

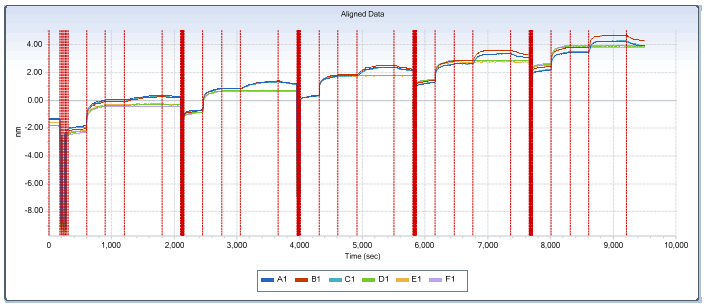

Figure 1b: *Processed Binding Curves and calculated kinetic rate constants (see* **Table 3***) for Biosensors A-C show very similar results.*

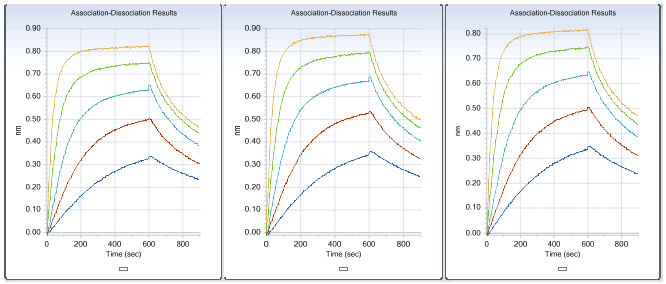

Table 3: *Calculated kinetic rate constants show good reproducibility.*

	kdis(1/s)	kon(1/Ms)	KD (M)
Biosensor A	1.91E-03	4.76E+05	4.00E-09
Biosensor B	1.91E-03	4.76E+05	4.01E-09
Biosensor C	1.81E-03	4.69E+05	3.86E-09

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