SARTORIUS

Octet® Anti-Mouse IgG Fc Capture (AMC) Biosensors

Technical Note

Scope
This technical note walks the reader through the optimal use of the Octet® AMC Biosensors for the kinetics characterization of the binding between mouse IgGs or mouse Fc region containing proteins and their binding partners.

Abstract

Octet® Anti-mouse IgG Fc capture (AMC) biosensors have a high affinity for the mouse Fc domain and can be used to immobilize any mouse Fc region containing molecule for the subsequent kinetics characterization of a target protein. The biosensor's specificity for mouse IgG Fc region containing molecules allows it to be used in cell culture and other crude matrix enabling a rapid method to perform kinetics experiments with mouse IgGs and related fusion proteins. In addition, it can be regenerated for re-use further allowing the user to cost save.

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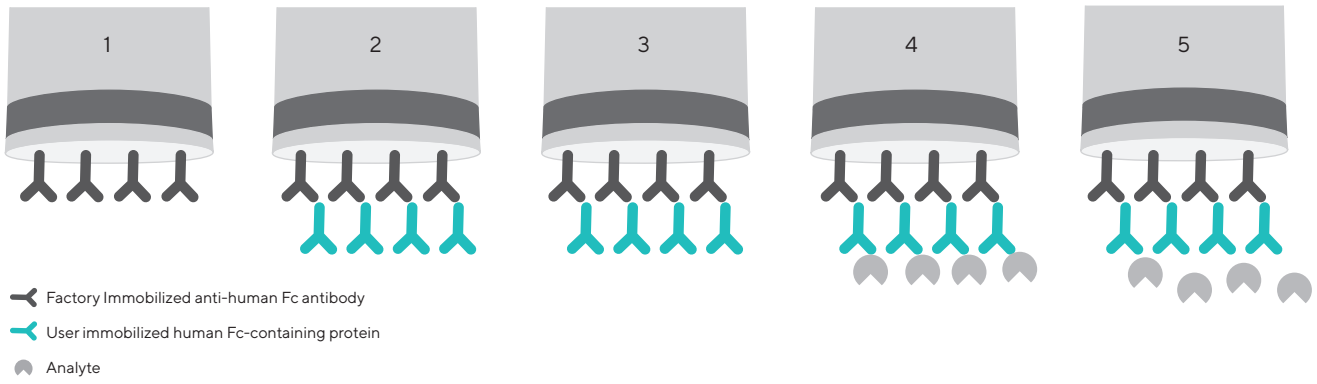
Overview

Octet® AMC Biosensors enable kinetic characterization of macromolecular interactions between mouse Fc-containing proteins and target analytes (Figure 1). Immobilization of mouse Fc-containing proteins is achieved through a factory immobilized anti-mouse Fc-specific antibody whose high affinity for the mouse Fc domain provides the stable baseline required for demanding kinetics applications. Cost-effective regeneration of the biosensors and the ability to directly immobilize mouse Fc-containing proteins from crude matrices make AMC Biosensors extremely useful in high-throughput applications.

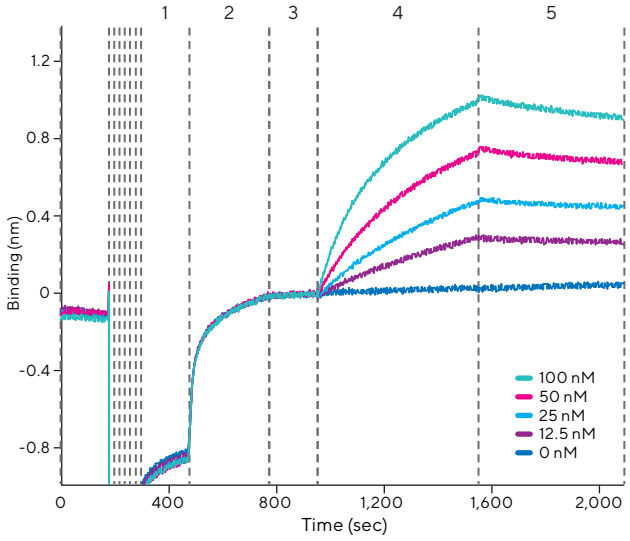
Figure 1

Kinetic Characterization of the Interaction Between a Mouse IgG1 Antibody and a FAb Analyte at 4 Different Concentrations Using AMC Biosensors.

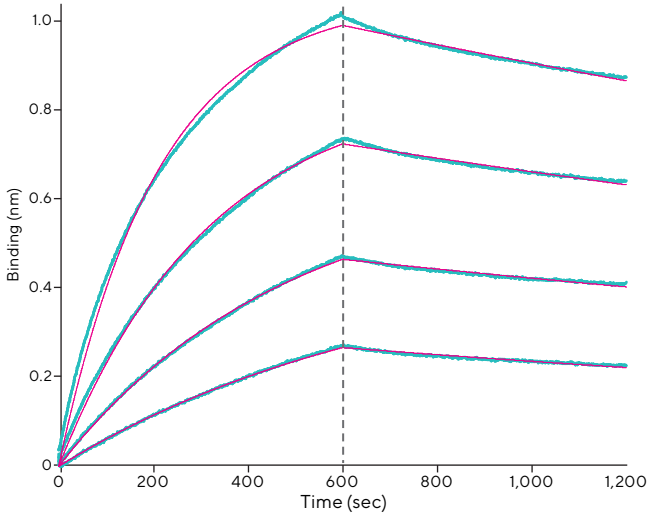
A.



B.



C.



D.

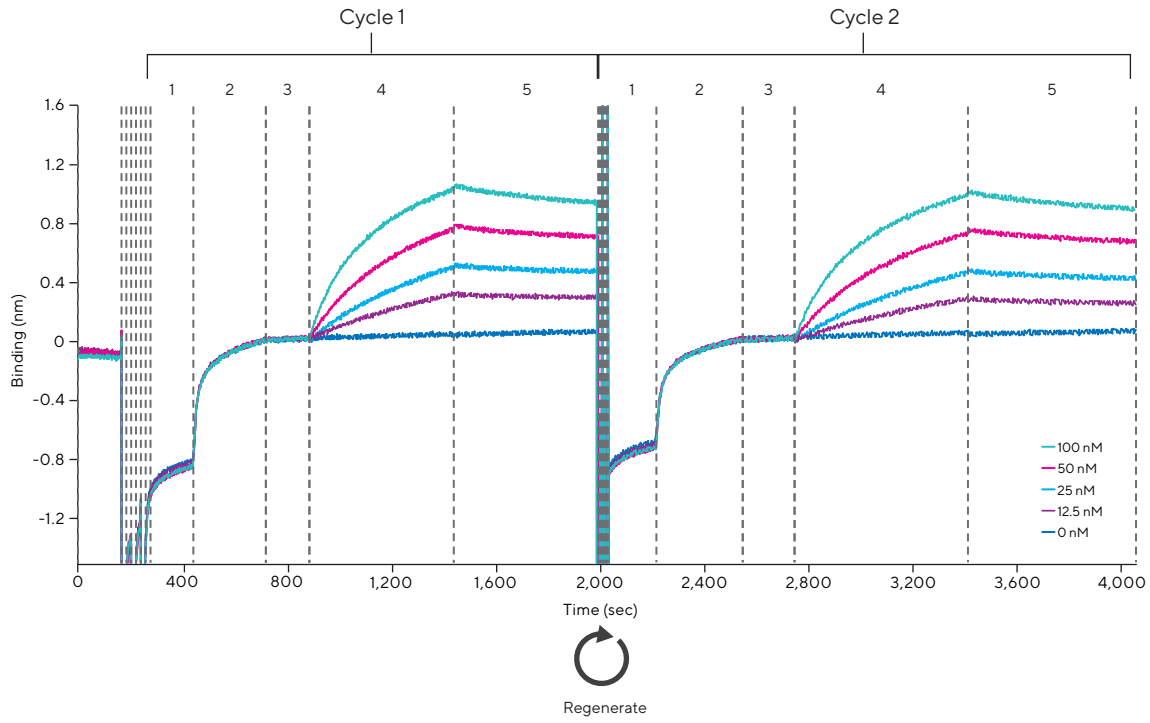
K_D (M)	k_{on} (1/Ms)	k_{on} Error	k_{dis} (1/s)	k_{dis} Error
5.34E-09	4.70E+04	1.60E+02	2.51E-04	1.78E-06

Note. After an equilibration step and a preconditioning cycle, the assay consists of 5 assay steps. Step 1: equilibration, Step 2: loading (capture) of mouse IgG1, Step 3: baseline, Step 4: association kinetics, Step 5: dissociation kinetics. The raw data for a full assay is shown in Fig 1B. After data processing (including reference subtraction using the 0 nM trace), the association and dissociation traces were fit to a 1:1 binding model (1C, red lines). Values of K_D, k_{on} and k_{dis} were extracted from the curve fitting analysis (1D).

Figure 2

Regenerated AMC Biosensors Deliver Precise Kinetic Results of the Interaction Between a Mouse IgG1 and a FAb antigen.

A.



B.

Cycle	K_D (M)	k_{on} (1/Ms)	k_{on} Error	k_{dis} (1/s)	k_{dis} Error
1	5.34E-09	4.71E+04	1.60E+02	2.51E-04	1.78E-06
2	5.59E-09	4.62E+04	1.55E+02	2.58E-04	1.76E-06

Note. After a pre-conditioning cycle, the interaction kinetics between a mouse IgG1 (captured ligand) and the FAb antigen (analyte) were determined using the five step assay described in Figure 1 (Figure 2B, Cycle 1). After 3 regeneration cycles at low pH (10 mM glycine pH 1.7), the assay was repeated (Figure 2B, Cycle 2). The kinetic results (K_D, k_{on} and k_{dis}) were highly comparable.

Principle

Octet® AMC Biosensors are pre-immobilized with a high-affinity antibody against the Fc portion of mouse IgG (mIgG). This antibody can capture and immobilize mIgG or other Fc-containing ligands to produce a stable surface suitable for interaction analysis. The capture surface is particularly suited for immobilizing mIgG from cell culture supernatants or other complex mixtures as an alternative to purifying the antibody and then biotinylating it.

AMC Biosensors can be regenerated up to 10 times via a standard low-pH protocol in as little as two minutes for select applications such as acquisition of replicate data (same ligand/analyte pair) and "bucket"-based screening applications (Figure 2).

Regeneration dissociates the mouse Fc-containing protein from the factory-immobilized anti-mouse Fc antibody, allowing additional analyses. For the highest quality affinity and kinetic results, using a new AMC Biosensor for each unique capture ligand is recommended.

Materials Required

- Octet® BLI system and Octet® Software
- Octet® AMC Biosensors (Sartorius part no. 18-5088 [tray]; 18-5089 [pack]; 18-5090 [case]
- For all Octet® BLI systems: 96-well, black, flat bottom, polypropylene microplate (Greiner Bio-One part no. 655209) and any other Sartorius approved sample plates
- Optional for Octet® RH16 and RH96 BLI systems:
  - 384-tilted well, black, flat bottom, polypropylene microplate (Sartorius part no. 18-5080 [pack]; 18-5076 [case])
  - 384-well, black, flat bottom, polypropylene microplate (Greiner Bio-One part no. 781209)
- Mouse IgG or other mouse Fc-containing proteins for immobilization. The mouse IgG can be dissolved in either buffer or a complex mixture such as culture supernatant.
- Analyte proteins that interact with immobilized mouse Fc-containing protein. The analyte proteins can be dissolved in buffer matrix or a complex mixture such as culture supernatant.

- Assay buffer. 1X Kinetics Buffer is recommended (dilute Octet® Kinetics Buffer 10X (10X KB) 1:10 with PBS, pH 7.4); other buffers can be used. Best results are obtained when all matrices are matched as closely as possible.
- Regeneration Buffer. 10 mM glycine pH 1.7 is required for surface conditioning and regeneration. Further pH optimization may be required.

Tips for Optimal Performance

- To improve baseline and surface stability, condition the biosensors before the first loading step. Typically, conditioning consists of three 20-second exposures to Regeneration Buffer alternating with 1X Kinetics Buffer.
- "Priming" the biosensor surface with a binding and regeneration cycle is recommended for the most rigorous applications. The priming binding cycle consists of capturing mIgG from solution and then regenerating the biosensors back to the original surface with the regeneration protocol. Biosensor priming should follow biosensor conditioning.
- Typically, the biosensor surface can be regenerated through five 5-second exposures to 10 mM glycine pH 1.7 alternating with 1X Kinetics Buffer. Depending on the mIgG, the regeneration protocol may require optimization.
- For optimal performance, a reference biosensor should be included. A reference biosensor should be loaded with the mIgG and should be run with a buffer blank for the association and dissociation steps.
- Equilibrate reagents and samples to room temperature prior to preparation. For frozen samples, thaw and mix thoroughly prior to use.
- Hydrating the biosensors is required prior to using them in an assay. Hydrating the biosensors in a buffer consistent with the buffer used throughout the assay will produce the most stable results.
- Ensure that the Octet® BLI system is turned on and the lamp is warmed up to room temperature for at least 40 minutes prior to starting the assay.
- Set the sample plate temperature in the Octet® Software by selecting File > Experiment > Set plate temperature and entering the desired temperature. Sartorius recommends assaying at 30 °C. Assaying at other temperatures may require different assay times than discussed in this protocol.

Assay Protocol

Overview

1. Prepare buffers, regeneration solution, ligand and interacting protein samples. Matching the hydration solution to the assay solution as closely as possible is recommended.
2. Hydrate the biosensors for at least 10 minutes.
3. Prepare the assay plate, filling columns of wells with buffers, regeneration solution, ligand and interacting protein samples.
4. Equilibrate both the hydrated biosensor assembly and the assay plate for 10 minutes on the Octet® BLI system. This allows the biosensors to fully hydrate and the assay plate to reach a stable temperature.
5. Run the assay.
6. Perform data processing and analysis.
7. Save the results.

Prepare Samples and Calibration Standards

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

- **Mouse IgG or other Fc-containing ligand:** The ligand is the protein that will be immobilized on the biosensor tip surface. A typical immobilization concentration for an Fc-containing ligand is 5–25 µg/mL. If the Fc-containing ligand is below 5 µg/mL, a longer loading time may be required (60 minutes loading at 1 µg/mL is equivalent to 10 minutes loading at 10 µg/mL). 200 µL/well, 80 µL/well and 40 µL/well of ligand solution are required for 96-well, 384-well and 384-well tilted-well plates, respectively. The ligand solution can be recovered from the well after the assay and re-used, if desired.
- If the ligand is to be captured from cell culture supernatants, dilution of the supernatant at least 1:2 with 1X Kinetics Buffer can potentially increase data quality. If dilution results in a low total concentration of the ligand, the biosensors can be incubated in the supernatant overnight at 4°C to maximize loading of the Fc-containing ligand. For tips on optimizing overnight loading, see Sartorius technical note "Batch Immobilization of a Biotinylated Ligand onto Streptavidin Biosensors".

- **Interacting protein (analyte):** During rigorous kinetic analysis, it is recommended to run a dilution series of at least four concentrations of the analyte protein. The highest concentration should be approximately 10-fold the expected KD. For example, concentrations of 90 nM, 30 nM, 10 nM and 3 nM would be recommended for an analyte with low-nanomolar affinity towards an immobilized ligand. 200 µL/well, 80 µL/well and 40 µL/well of analyte solution are required for 96-well, 384-well and 384-well tilted-well plates, respectively. The solution can be recovered from the well after the assay and re-used, if desired.
- For screening assays or qualitative interaction analysis, a single concentration of the interacting protein can be sufficient to characterize the binding.
- **Conditioning|regeneration solution:** The capture mouse Fc-containing ligand and the analyte can be removed from the biosensors by exposing them to 10 mM glycine pH 1.7 followed by a neutralization buffer (typically 1X Kinetics Buffer). 200 µL/well, 80 µL/well and 40 µL/well of regeneration and neutralization solutions are required for 96-well, 384-well and 384-well tilted-well plates, respectively. After regeneration, the biosensor can be re-loaded with mIgG for a new interaction analysis. A small loss in binding capacity after a regeneration cycle is expected. Regeneration provides a cost-effective format for generating replicate data for ligand-analyte pairs. For the highest quality kinetic results, using a new biosensor to capture each unique ligand is recommended.

Hydrate Biosensors and Prepare Assay Plate

1. Pipette 200 µL/well of biosensor hydration solution into wells of a 96-well black, flat-bottom microplate corresponding to the number and positions of biosensors to be used.
2. Insert the hydration plate into the biosensor tray. Align the biosensor rack over the hydration plate and lower the biosensors into the wells, taking care not to scrape or touch the bottom of the biosensors. Allow biosensors to hydrate for a minimum of 10 minutes (it is critical to hydrate the biosensors for at least 10 minutes).
3. Transfer 200 µL of each assay reagent into the appropriate wells of a black polypropylene microplate. A sample plate map for a typical assay performed in a 96-well microplate is shown in Figure 3.

Load Proteins on Biosensors

Table 1 lists typical assay steps used to load biosensors online (i.e., on the Octet® BLI system). These steps are fully automated by the Octet® BLI System.

1. Place the assay plate on the sample plate stage with well A1 toward the back right corner.
2. Place the biosensor hydration assembly in the Octet® BLI system on the left stage. Ensure that both the biosensor tray and sample plate are securely in place.

Prepare Instrument and Run Experiment

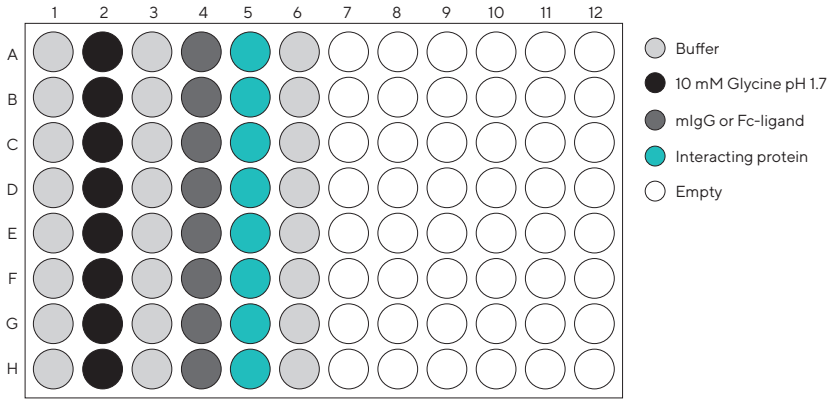
1. Ensure that the Octet® BLI system and computer are turned on. It is essential that the instrument lamp warms up for at least 40 minutes before running an experiment.
2. Equilibrate the plates in the instrument for 10 minutes prior to starting the experiment. The delay timer can be used to automatically start the assay after 10 minutes (600 seconds).
3. Set up a kinetic assay. For details, see the Octet® BLI Discovery User Guide. Table 2 shows an example kinetic assay including a pre-conditioning cycle, ligand loading, association, dissociation and regeneration steps, using the plate described in Figure 3.
4. Run the assay.

Process and Analyze the Data

1. Load data into the Octet® Analysis Studio software.
2. Process the data by specifying methods for reference subtraction, y-axis alignment, inter-step correction and Savitzky-Golay filtering. For details on each processing parameter, refer to the Octet® Analysis Studio User Guide.
3. Analyze the data by specifying steps for analysis, fitting method (local or global) and window of interest. For details on each analysis parameter, refer to the Octet® Analysis Studio User Guide.
4. To export the analyzed data, use the Save Report button to generate a Microsoft® Excel® report. For details on data exporting, refer to the Data Analysis User Guide.

Figure 3

Example Plate Map for a Kinetic Assay that iIncludes a Pre-conditioning cycle, Ligand Loading, Association, Dissociation and a Regeneration Cycle.



Note. An assay step list using the sample plate is described in Table 2. The same sample wells (column 3) are used for the baseline and dissociation steps for optimal use of the inter-step correction processing feature.

Table 1

Typical Steps used in a Kinetic Assay with Regeneration.

Step #	Step Function
1	Equilibrate (typically in 1X Kinetics Buffer or media sample)
2	Condition (recommended). Expose the biosensor to one regeneration cycle (three repetitions of glycine pH 1.7 followed by neutralization) to optimize baseline performance and to stabilize the surface density.
3	Load ligand for priming binding (optional)
4	Regenerate for priming binding (optional)
5	Neutralize for priming binding (optional)
6	Load ligand (in 1X Kinetics Buffer or media sample)
7	Baseline (in 1X Kinetics Buffer or media sample)
8	Association of the interacting protein (1X Kinetics Buffer or media sample)
9	Dissociation (in 1X Kinetics Buffer or media sample)
10	Regenerate(optional) (in 10 mM glycine pH 1.7, optional)
11	Neutralize (optional) (in 1X Kinetics Buffer or media sample)

Priming binding cycle

Table 2

Example Assay Steps and Associated Parameters.

Step #	Step Name	Time (Sec)	Flow (rpm)	Step Type	Sample Plate Column (See Figure 3)
1	Equilibration	180–600	1000	Custom	1
2	Conditioning (10 mM glycine)	20	1000	Custom	2
3	Neutralization	20	1000	Custom	1
4	Conditioning (10 mM glycine)	20	1000	Custom	2
5	Neutralization	20	1000	Custom	1
6	Conditioning (10 mM glycine)	20	1000	Custom	2
7	Neutralization	20	1000	Custom	1
8	Equilibration	300–600	1000	Custom	3
9	Loading	300–600	0-1000	Loading	4
10	Baseline	180–600	1000	Baseline	6
11	Association	600–1800	1000	Association	5
12	Dissociation	600–3600	1000	Dissociation	6
13	Regeneration (10 mM glycine)	5	1000	Custom	2
14	Neutralization	5	1000	Custom	1
15	Regeneration (10 mM glycine)	5	1000	Custom	2
16	Neutralization	5	1000	Custom	1
17	Regeneration (10 mM glycine)	5	1000	Custom	2
18	Neutralization	5	1000	Custom	1
19	Regeneration (10 mM glycine)	5	1000	Custom	2
20	Neutralization	5	1000	Custom	1
21	Regeneration (10 mM glycine)	5	1000	Custom	2
22	Neutralization	5	1000	Custom	1

Note. After regeneration, steps 8–22 would be repeated to reload and perform the next assay. The same sample wells (column 3) are used for the baseline and dissociation steps for optimal use of the inter-step correction processing feature.

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