SARTORIUS

Octet® AMC2 Biosensors

For Quantitation and Kinetic Characterization of Murine IgG Antibodies

Technical Note

Scope
This Technical Note describes kinetic and quantitation assay workflows that use the Octet® AMC2 Biosensors for characterization of murine IgG proteins.

Keywords or phrases:
Octet®, Bio-Layer Interferometry, BLI, Kinetic analysis, Quantitation, Murine IgG, mIgG, Antibody capture, Biosensor regeneration.

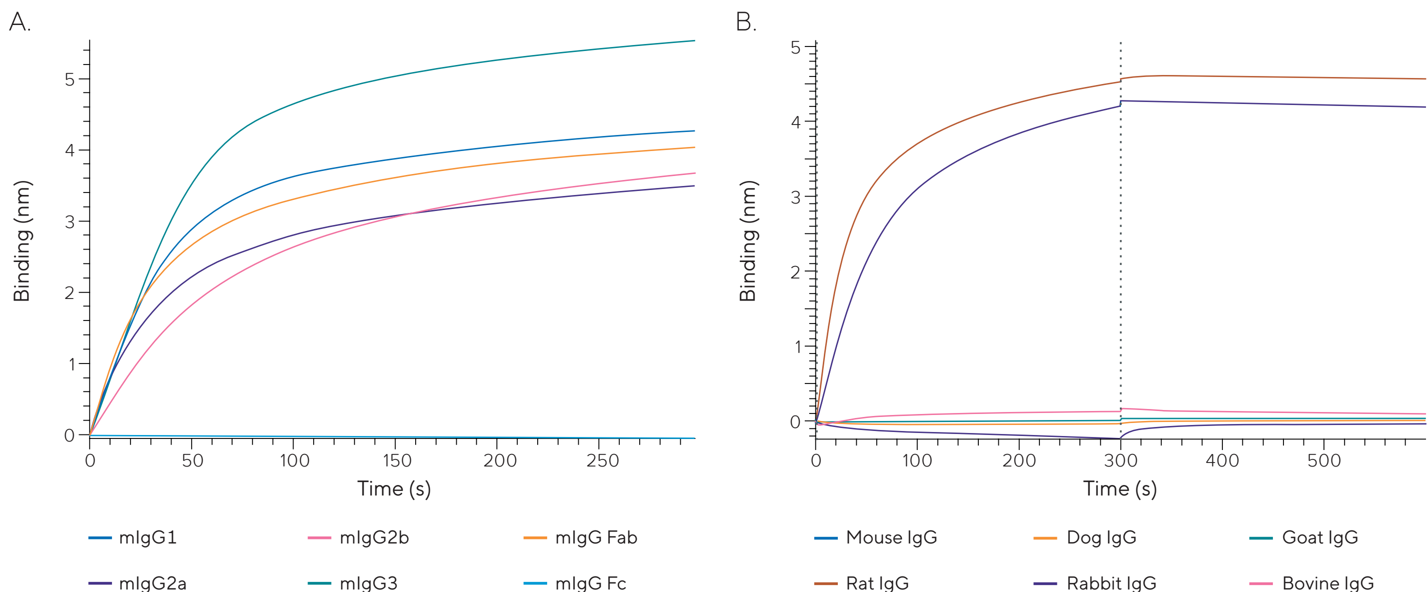
Introduction

Mouse IgG antibodies are used for a broad range of applications and most commonly for hybridoma screening during therapeutic antibody discovery. To expedite the antibody discovery process it is essential to have a rapid and robust method for quantitation and kinetic characterization of these proteins. The Octet® Bio-Layer Interferometry (BLI) Platform in combination with Octet® Anti-Mouse IgG Fc Capture (AMC) Biosensors have been widely used for this application as they offer an easy, label-free, and high-throughput characterization of antibodies in both purified and cell culture samples. To further enhance the quantitation and kinetic analysis of mouse and rat IgG proteins, Sartorius has developed the second-generation Octet® AMC2 Biosensors. These biosensors enable both quantitation and kinetic characterization and feature increased ligand binding capacity, wide dynamic range and improved regenerability for more efficient and cost-effective murine IgG screening on the Octet® BLI Platform.

The Octet® AMC2 Biosensors are highly specific to four mouse IgG (IgG1, IgG2a, IgG2b and IgG3) and rat IgG (IgG1, IgG2a, IgG2b and IgG2c) subclasses and do not interact with IgGs from other species, such as bovine, rabbit, goat, sheep IgG (Figure 1). These biosensors bind to the constant domain of kappa light chain on mIgGs (not the Fc-region) and can be used for quantitation and kinetic characterization of mIgG in

both crude and purified samples. In addition, cost-effective regeneration of the AMC2 biosensors up to 10 times makes them an extremely useful solution for high-throughput applications, such as lead identification and optimization, cell line development, process development and QC. This Technical Note describes in detail kinetic and quantitation assay workflows that use the Octet® AMC2 Biosensors for characterization of murine IgG proteins.

Figure 1: *Specificity of the Octet® AMC2 Biosensors.*

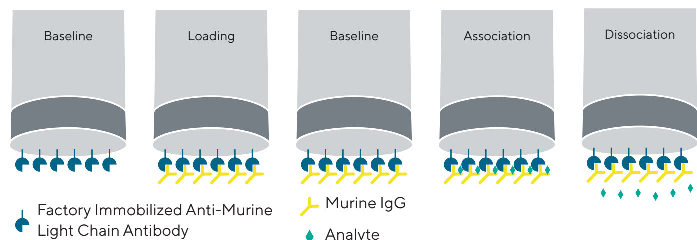


Note: (A) Strong binding of AMC2 to all mIgG subtypes, IgG1, IgG2a, IgG2b, IgG3 and IgG Fab fragment, no binding of AMC2 to the mIgG Fc fragment. (B) Strong binding to Mouse and Rat IgGs, no binding to IgGs from dog, rabbit, goat, bovine.

Kinetic Assay Workflow

The Octet® AMC2 Biosensors are pre-immobilized with an antibody that specifically binds to the constant domain of kappa light chains of mouse and rat antibodies, which enables the immobilization of murine IgG directly from a crude or purified matrix. The AMC2 biosensors provide up to 3-fold increased binding capacity for mIgG when compared to the previous generation of the AMC biosensors, making the second generation particularly suitable for high sensitivity kinetic assays such as analysis of proteins at low concentrations or characterization of low molecular weight protein analytes. An example of assay workflow utilizing the Octet® AMC2 Biosensors to characterize the interaction between an analyte and a mIgG is outlined in Figure 2.

Figure 2: *Kinetic Assay Workflow Using the Octet® AMC2 Biosensors*



Note: 1 – equilibration (baseline), 2 – loading (capture) of mIgG, 3 – baseline, 4 – association phase, 5 – dissociation phase.

Materials Required

- Octet® BLI system with Octet® BLI Discovery and Octet® Analysis Studio software
- Octet® AMC2 Biosensors (Sartorius part no. 18-5163 (tray), 18-5164 (pack), 18-5165 (case))
- For all Octet® BLI systems: 96-well, black, flat bottom microplate (Greiner Bio-One part no. 655209)
- Optional for Octet® RH16 and RH96 BLI systems:
  - Octet® 384-Well Tilted bottom Plate (Sartorius part no. 18-5080 (pack); 18-5076 (case))
  - 384-well, black, flat bottom, polypropylene microplate (Greiner Bio-One part no. 781209)
- mIgG for immobilization. The mIgG can be present in either buffer or a complex mixture such as cell culture supernatant.
- Analyte protein that interacts with mIgG. The analyte proteins can be dissolved in a buffer solution or a complex mixture such as cell culture supernatant. The buffer matrix of the analyte should be identical to the baseline buffer immediately prior to the association step, where the concentrations of the bulk components of the baseline buffer and the analyte buffer are the same.
- Assay buffer. Octet® 10X Kinetics Buffer (Sartorius part no. 18-1105) or 1X Kinetics Buffer (dilute 10X Kinetics buffer 1:10 with PBS, pH 7.4) are recommended, other buffers can also be used. Best results are obtained when all matrices are matched as closely as possible.

Assay Optimization Tips

- Use a reference sample to correct for baseline drift. A reference sample is run on a biosensor that has ligand present in the loading step but with zero analyte in the association step, i.e. a buffer-only negative control. When using a capture-based biosensor such as AMC2, some background level of dissociation of the captured IgG ligand from the sensor will occur. This background dissociation, or assay drift, can be subtracted out using the reference sample.
- The Baseline step right before Association and the Dissociation step should be performed in the same microplate well for each biosensor. This enables the inter-step correction feature to align the Association and Dissociation steps when processing data.

Assay Procedure

For details on setting up a kinetic assay in Octet® software, please refer to the Octet® BLI Discovery Software User Guide. Figure 3 shows an example microplate layout and assay design for a kinetic characterization assay using the Octet® AMC2 Biosensors. For all steps, use a 200 µL sample volume for 96-well plates, 80 µL for standard 384-well plates and 40-80 µL for tilted 384-well plates.

Figure 3: *(A) Sample Plate Map and (B) Assay Steps With Associated Parameters for the AMC2 Kinetic Assay.*

A.

	1	2	3	4	5-12
A	mIgG buffer	mIgG (1-25µg/mL)	Analyte Sample buffer	Analyte	
B	mIgG buffer	mIgG (1-25µg/mL)	Analyte Sample buffer	Analyte	
C	mIgG buffer	mIgG (1-25µg/mL)	Analyte Sample buffer	Analyte	
D	mIgG buffer	mIgG (1-25µg/mL)	Analyte Sample buffer	Analyte	
E	mIgG buffer	mIgG (1-25µg/mL)	Analyte Sample buffer	Analyte	
F	mIgG buffer	mIgG (1-25µg/mL)	Analyte Sample buffer	Analyte	
G	mIgG buffer	mIgG (1-25µg/mL)	Analyte Sample buffer	Analyte	
H	mIgG buffer	mIgG (1-25µg/mL)	Analyte Sample buffer	Reference Sample	

B.

Step #	Column #	Description	Step type	Time	Shaking speed (rpm)
Step 1	1	Equilibration buffer	Custom	180	1000
Step 2	2	Loading of mIgG	Loading	120–600	1000
Step 3	3	Baseline in Analyte sample buffer	Baseline	300–600	1000
Step 4	4	Association of Analyte	Association	300–900	1000
Step 5	3	Dissociation of Analyte	Dissociation	300–3600	1000

Before the Assay –
Warm up all reagents and samples to room temperature before biosensor hydration and assay setup. Pre-hydration of biosensors: Hydrate the AMC2 biosensors in 200 µL per well of similar matrix as mIgG to be captured. Hydration is performed in a 96-well, black, flat-bottom plate (Greiner 655209) for a minimum of 10 minutes.

Assay Step 1 – On-line equilibration of the hydrated AMC2 biosensors in 10X Kinetics Buffer or mIgG custom buffer: Add buffer, media, or diluted lysate to Column 1 of the Sample Plate according to the map in **Figure 3.** Note the equilibration buffer should match the buffer matrix of mIgG to be captured.

Assay Step 2 – Capture of mIgG or Fab-containing protein (Loading/Immobilization): Dilute the mIgG or Fab-containing protein to the appropriate concentration in 10X Kinetics Buffer or the corresponding sample matrix and add the solution to the Sample Plate. The matrix or buffer used should typically match the one used for equilibration in Assay Step 1. The typical immobilization concentration is 1-25 µg/mL and should be optimized for each interaction being measured. The concentration of ligand to use will depend on its affinity for the associating analyte, as well as the size of both ligand and analyte.

For the best kinetic data and most accurate affinity constants, a loading optimization experiment should be performed to determine the optimal ligand loading concentration and time. Load only enough ligand so that the highest concentration of analyte used has adequate association signal at equilibrium and allows measurement of the dilution series. Loading more ligand than what is needed can cause artifacts such as non-specific binding, heterogeneity, or mass transport. Loading optimization is recommended to define the optimal ligand density, and for more details please refer to the following Application Notes: 1) **Biomolecular Binding Kinetics Assays on the Octet® BLI Platform** and 2) Octet® AMC2 Biosensors Application Note.

Assay Step 3 – Baseline step in assay buffer (Baseline): Add 10X Kinetics Buffer or alternative buffer matching the analyte samples being analyzed to the Sample Plate according to Figure 2. It is important to match the baseline buffer matrix to that of the analyte samples, where the concentrations of the bulk components of the baseline buffer and the analyte buffer are the same. The baseline step should be run for a long enough time to allow for any change in baseline drift to stabilize. We recommend 300-600 seconds of baseline if a new buffer matrix is used in this step. If the buffer is identical to the mIgG ligand buffer, a baseline step of 120-300 seconds should be adequate.

Assay Step 4 – Association to interacting analyte (Association): If detailed kinetic characterization is being performed, the analyte protein must be purified and of known concentration. It is recommended to run a titration series of at least four to five concentrations of the analyte protein and perform a global fitting of all concentrations to determine k_a, k_{dis}, and K_D values. The highest analyte concentration should be greater than 10 times the expected K_D. For screening assays or qualitative interaction analysis, a single concentration of the interacting protein can be sufficient to characterize the binding. Analyte samples must be diluted in the same buffer used for the baseline and dissociation steps. Include a reference sample, consisting of assay buffer blank with no analyte present, in this step to enable subtraction of background baseline drift.

Assay Step 5 – Dissociation of interacting analyte (Dissociation): The Dissociation step is performed in the same buffer well(s) used for the Baseline step (step3). Using the same wells for Baseline and Dissociation enables the inter-step correction feature to be used in data analysis for more accurate curve fitting.

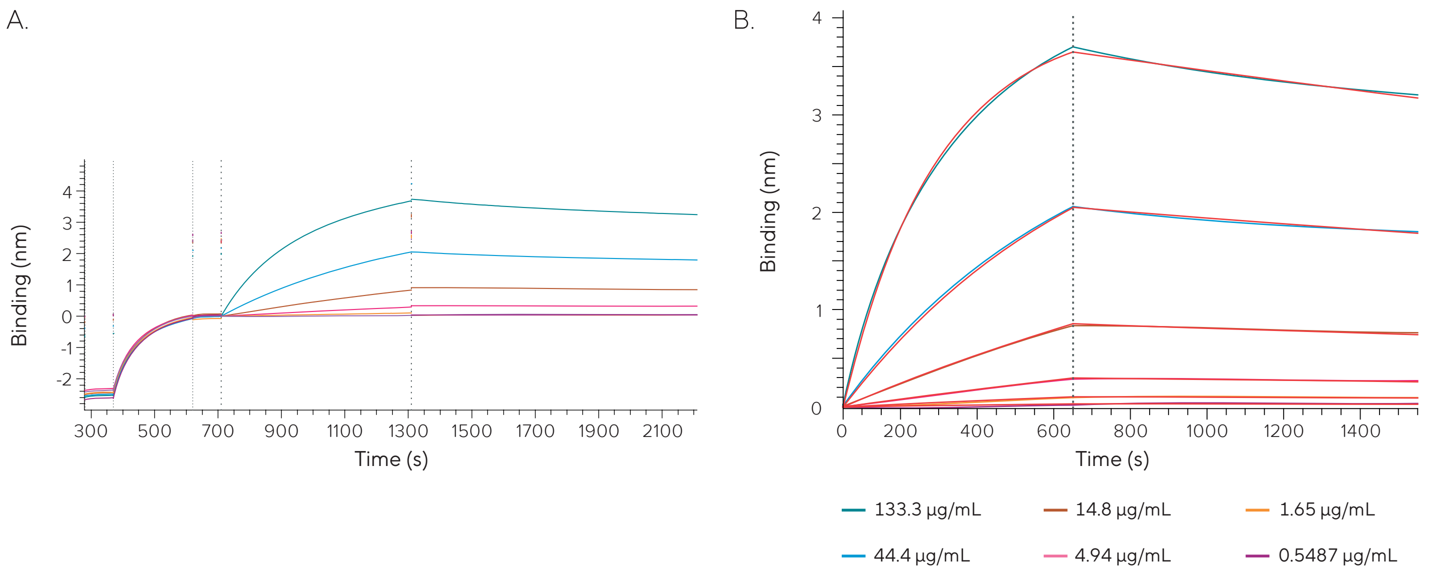
Process and Analyze Data

1. Load data into the Octet® Analysis Studio software.
2. Process the data by specifying methods for reference subtraction, y-axis alignment by baseline, inter-step correction by dissociation and check the Savitzky-Golay filtering.
3. Analyze the data by specifying steps for analysis, fitting method (1:1 binding, global fit) and time window of interest.
4. To export the analyzed data, click Save Report to generate an excel report.

Representative Data

Figure 4 shows AMC2 biosensors were loaded with mIgG1 (Millipore Sigma part no. M9269) at 10 µg/mL and followed by kinetic analysis of an analyte AffiniPure Fab Fragment Goat Anti-Mouse IgG (H+L) (50 kDa) (Jackson Immuno Research part no. 115-007-003).

Figure 4: *Binding Kinetics of mIgG1 (150 kDa) And an Analyte Fab Fragment, Goat Anti-mIgG (H+L) Specific (50 kDa), on the Octet® BLI Platform.*



Note: (A) Loading of mIgG1 at 10 µg/mL and followed by kinetic analysis of an analyte, Fab fragment Goat Anti-Mouse IgG (H+L) (50 kDa). (B) Association-dissociation curves for the interaction between the mIgG1 and the Fab fragment. 1X Kinetics Buffer was used as a buffer matrix throughout the assay. The association and dissociation traces were fit to a 1:1 binding model. The kinetic results are reported in Table 1.

Table 1: Kinetic results for the interaction between the ligand mIgG1 (150 kDa) and an analyte Fab fragment, goat anti-mIgG (H+L) specific (50 kDa), using the Octet® AMC2 Biosensors for the data shown in Figure 4.

k_a (1/Ms)	k_{dis} (1/s)	K_D (M)
2.95E+04	1.55E-04	5.25E-09

Quantitation Assay Workflow

The Octet® AMC2 Biosensors have high specificity towards all four mIgG subclasses (IgG1, IgG2a, IgG2b and IgG3) and can be used to quantitate both crude and purified mIgG with concentrations in the range of 0.025 – 8,000 µg/mL depending on the assay conditions. For mIgG samples with concentration range of 0.5 – 8,000 µg/mL, it is recommended to use shaking speed of 400 RPM with 1-min assay read time. However, if mIgG concentration is in the range of 0.025–0.5 µg/mL, it is typically recommended to use 96-well plate as a sample plate at shaking speed of 1,000 RPM and a longer 10-min assay read time for improved sensitivity.

Materials Required

- Octet® BLI systems with Octet® BLI Discovery and Analysis Studio software
- Octet® AMC2 Biosensors (Sartorius part no. 18-5163 (tray), 18-5164 (pack), 18-5165 (case))
- For all Octet instruments: 96-well, black, flat bottom microplate (Greiner Bio-One part no. 655209)
- Optional for Octet® RH16 and RH96 BLI systems:
  - Octet® 384-tilted well, black, flat bottom, polypropylene micro- plate (Sartorius part no. 18-5080 (pack); 18-5076 (case))
  - 384-well, black, flat bottom, polypropylene microplate (Greiner Bio-One part no. 781209)
- Purified standard protein (that is of the same molecule as the unknown samples) to be used as a calibration standard.
- Octet® Sample Diluent (Sartorius part no. 18-1104) for dilution of all samples. If undiluted crude samples are to be quantified, a blank buffer (that is free of the molecules of interest) with the same matrix is required.

Assay Optimization Tips

The following optimization steps are recommended each time when the quantitation assay involves a new matrix or new mIgG protein.

- The calibration standard should be identical to the molecule present in the unknown sample for best results.
- Concentrations of the calibration standards should cover the range of concentrations in the unknown samples.
- Match the matrix of the samples, standards, references, and hydration solution as closely as possible.
- Use a blank negative control in a matching matrix for background signal subtraction. This is especially important when optimizing accuracy and detecting low-concentration analytes.
- Determine the minimal dilution factor required to achieve the targeted assay performance.
- Perform a spike/recovery study to determine the assay dynamic range.
- Qualify the reagents and buffers used in assays routinely and use best laboratory practice to aliquot and store reagents and samples.
- Establish data analysis parameters in Octet® Analysis Studio software.
- Apply the finalized protocol and data analysis parameters in routine assays.

Dilution Factor Determination for Sample Matrix

Components in complex matrices such as cell culture media can potentially interfere with assay performance. Diluting the sample matrix using the Octet® Sample Diluent is an effective means of minimizing matrix effects. Dilution factor guidelines for various sample types are described in Table 2. However, before running a quantitation assay it should be empirically determined whether dilution of samples is needed.

1. Prepare 1 mL of each sample matrix (without target protein) diluted both 2-fold and 10-fold in the Octet® Sample Diluent.

2. Add target protein to each of the matrix dilutions, and also to neat matrix and to Sample Diluent as a control. The final concentration of target protein in each of the four samples should be in the middle of the desired quantitation range.

3. Transfer each sample to a 96- or 384-well sample plate in duplicate (eight wells total).

4. Hydrate biosensors in the sample matrix that matches each sample type (e.g., biosensors to be used in wells with 10-fold diluted matrix should be hydrated in 10-fold diluted matrix).

5. Set up a Basic Quantitation assay according to the Octet® BLI Discovery Software User Guide.

6. Run the assay.

Table 2: Recommended minimum dilution for common sample types. In all cases the matrix for the diluted samples, the standards and the biosensor hydration solution should be matched as closely as possible

Sample type	Minimum recommended dilution in sample diluent buffer
mIgG in CHO media	Neat
mIgG in DMEM	2-fold
mIgG in DMEM with 10% FBS	Neat
mIgG in PBS	2-fold
mIgG in Octet® Sample Diluent	Neat

Recovery and Precision Assay to Determine Quantitation Range

1. Prepare a series of protein standards in the appropriate matrix diluent using the dilution factor determined in the Dilution Factor Determination for Sample Matrix experiment. The dilution series should span the entire range of the assay based upon user experimental goal, such as 0.5–8000 µg/mL for assays run at 400 RPM.

2. Using the same matrix diluent as in Step 1, prepare two protein samples of known concentration for recovery measurement. The concentration of these samples should be within the range of the standard curve being generated, preferably one at the low end and one at the high end. These will be defined as unknown samples in the assay for calculating recovery.

3. Transfer triplicates of the prepared standards and the samples to a sample plate. It is recommended to organize samples from row A–H. Fill at least one well with blank diluted matrix for reference subtraction during data analysis. An example plate map is shown in Figure 5.

4. Hydrate biosensors for 10 minutes in matching matrix diluent.

5. Set up a Basic Quantitation assay using the same assay parameters that were used in the Dilution Factor Determination for Matrix experiment. Define sample Replicate Groups in order to calculate replicate averages and %CVs.

6. Run the experiment. Data will be displayed in real time during the assay. Data files, method files and assay pictures will be saved automatically.

7. Load the data into Octet® Analysis Studio software.

8. If a blank matrix was included as a reference, use the reference subtraction option to correct the data as appropriate.

9. Calculate the binding rate. The results table will populate with calculated concentrations and data statistics.

10. Define assay dynamic range by selecting acceptable %CV values for the lower and upper concentration limits in the standard curve.

11. Exclude data points for the standard curve that lie outside the defined dynamic range if necessary.

12. Select the appropriate equation to fit the standard curve. Start the fitting with a 4- or 5-parameter logistic model, although other models may be used with suitable validation. Use a 5-parameter logistic equation for better recovery results if there are more lower concentration data points.

13. Evaluate the accuracy and precision of the assay using calculated concentration value of the unknowns to determine % recovery and %CV.

Figure 5: *Example Plate Layout for a Spike Recovery Assay.*

	1	2	3	4	5-12
A	mIgG Standard	mIgG Standard	mIgG Standard	mIgG Sample 1	
B	mIgG Standard	mIgG Standard	mIgG Standard	mIgG Sample 1	
C	mIgG Standard	mIgG Standard	mIgG Standard	mIgG Sample 1	
D	mIgG Standard	mIgG Standard	mIgG Standard	mIgG Sample 2	
E	mIgG Standard	mIgG Standard	mIgG Standard	mIgG Sample 2	
F	mIgG Standard	mIgG Standard	mIgG Standard	mIgG Sample 2	
G	mIgG Standard	mIgG Standard	mIgG Standard	Reference sample	
H	mIgG Standard	mIgG Standard	mIgG Standard	Reference sample	

Running the Assay to Quantify Proteins of Interest

1. Prepare samples, calibration standards and hydration solution according to the conditions determined in optimization steps in the prior section.

2. Set up a Basic Quantitation assay using the parameters described previously in the optimization experiments. See Figure 6 for an example assay set up. Please note that standards can be run across the plate and not all at the beginning.

3. Run the assay.

4. Load data into Octet® Analysis Studio software. Analyze as in previous optimization steps to determine concentration of samples and data statistics.

5. To export the analyzed data, click Save Report to generate a Microsoft Excel report.

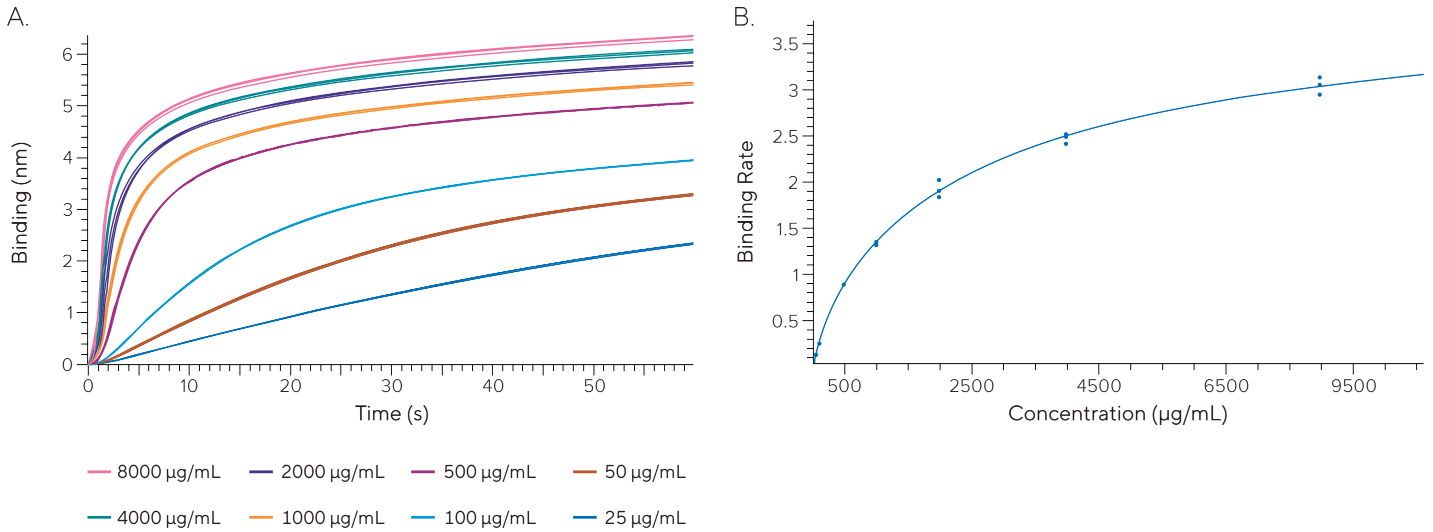
Figure 6: *Example Plate Layout for a Routine Quantitation Assay Run in a 96-Well Microplate.*

	1	2	3	4	5	6	7	8	9	10	11	12
A	mIgG Standard	mIgG Standard	mIgG Standard	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample
B	mIgG Standard	mIgG Standard	mIgG Standard	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample
C	mIgG Standard	mIgG Standard	mIgG Standard	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample
D	mIgG Standard	mIgG Standard	mIgG Standard	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample
E	mIgG Standard	mIgG Standard	mIgG Standard	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample
F	mIgG Standard	mIgG Standard	mIgG Standard	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample
G	mIgG Standard	mIgG Standard	mIgG Standard	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	Reference sample
H	mIgG Standard	mIgG Standard	mIgG Standard	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	mIgG Sample	Reference sample

Representative Data

Figure 7 shows the detection of mIgG using the AMC2 biosensors on the Octet® R8 BLI system. A standard curve was run to demonstrate quantitation of mIgG with triplicates at concentration range of 25-8,000 µg/mL.

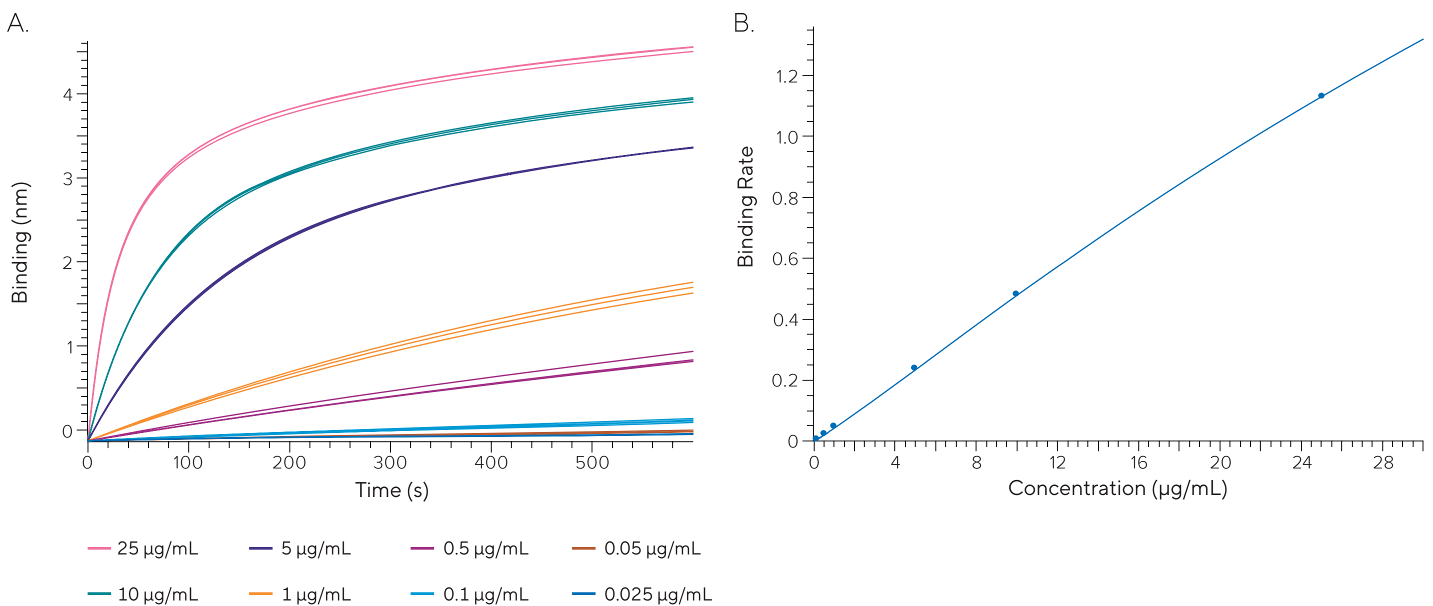
Figure 7: *Quantitation of mIgG Using Octet® AMC2 Biosensors*



Note: (A) mIgG dose response for concentrations within the dynamic range of 25–8000 µg/mL on the Octet® R8 instrument with assay parameters: 400 rpm shake speed, 1 min assay read time. (B) mIgG standard calibration curve generated from triplicate samples and calculated using 4PL fitting model. The Octet® Sample Diluent Buffer was used as a matrix for all samples.

When the sample concentration is in the range of 0.025 - 0.5 µg/mL, it is highly recommended to use 96-well plate as sample plate, set 1,000 rpm as shaking speed and a longer 10-min assay time for increased sensitivity. Figure 8 shows the detection of mIgG at low concentration using the AMC2 biosensors on the Octet® R8 BLI system.

Figure 8: *Quantitation of mIgG Using Octet® AMC2 Biosensors*



Note: (A) mIgG dose response for concentrations within the dynamic range of 0.025 – 25 µg/mL on the Octet® R8 system with assay parameters: 1000 rpm shake speed, 10 min assay read time. (B) mIgG standard calibration curve generated from triplicate samples and calculated using 4PL fitting model. The Octet® Sample Diluent Buffer was used as a matrix for all samples.

Table 3: Calculated concentrations, %CV and %Recovery for mIgG (0.025-8000 µg/mL) quantitation assay with triplicates.

Known Conc. (µg/mL)	Well Conc. (µg/mL)	%CV (n=3)	%Recovery
8000	8073.09	10%	101%
4000	3956.42	7%	99%
2000	2067.21	10%	103%
1000	977.69	6%	98%
500	505.05	1%	101%
100	100.39	1%	100%
50	49.62	1%	99%
25	25.00	1%	100%
10	10.000	1%	100%
5	5.005	3%	100%
1	0.995	4%	100%
0.5	0.506	6%	101%
0.1	0.099	1%	99%
0.05	0.051	3%	102%
0.025	0.025	10%	100%

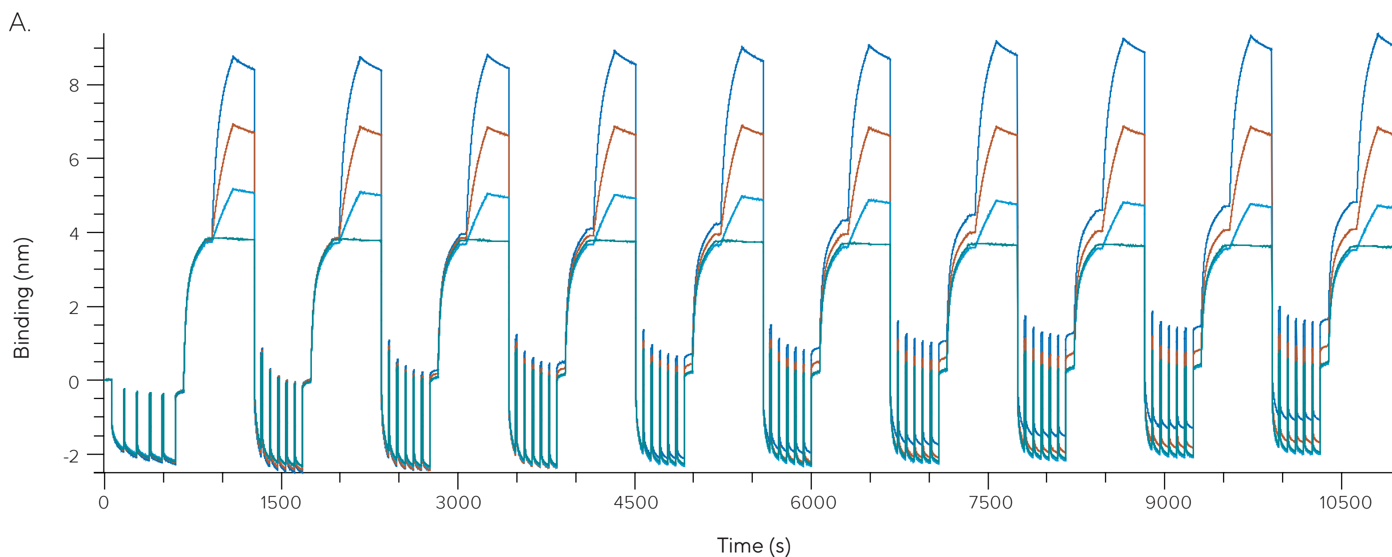
Regeneration of Octet® AMC2 Biosensors

The Octet® AMC2 Biosensors can be cost-effectively regenerated and re-used up to 10 times in both kinetic and quantitation assays for generating replicate data for ligand-analyte pairs, or for analyzing large numbers of samples in sequence. The regeneration of the Octet® AMC2 Biosensors is performed by dipping the biosensors into a solution of 10~100 mM Phosphoric acid solution, for 60 seconds, followed by a dip in the assay buffer for 5 seconds. These regeneration steps should be repeated 3 – 5 times in sequence to fully remove bound mIgG or the interaction complex. After regeneration, the biosensor can be immobilized with mIgG for a new analysis. For best results it is recommended to pre-condition biosensors by running the regeneration protocol prior to loading the ligand the first time. When optimizing the regeneration condition for the Octet® AMC2 Biosensors, Glycine solution is not recommended.

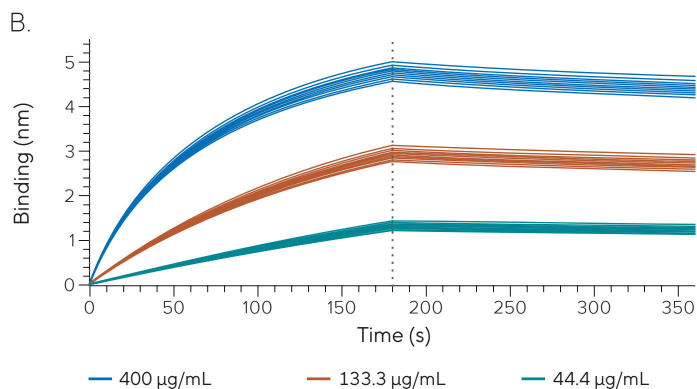
Regeneration results will depend on the captured molecule and a small loss in binding capacity may occur after each regeneration cycle. The exact number of possible regenerations should be determined experimentally and will depend on assay precision requirements. See examples of kinetics and quantitation assays with various regeneration cycles in Figures 9 and 10, Tables 4 and 5.

Figure 9: *Regeneration Assessment of AMC2 Biosensors for Kinetics Assays.*

A.



B.

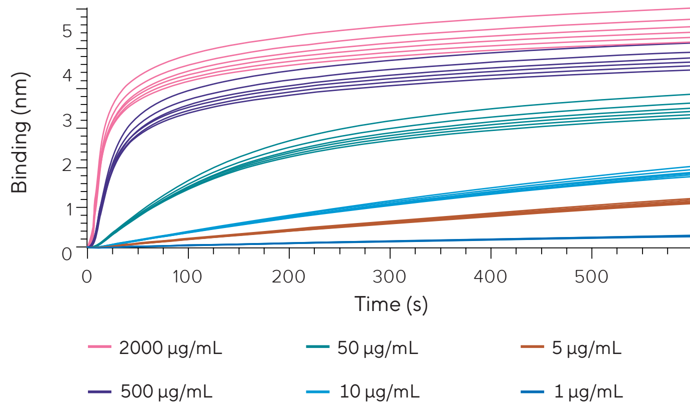


Note: (A) Binding of AMC2 captured mIgG to mIgG Fab protein over 10 regeneration cycles using 10 mM Phosphoric acid. (B) Overlay of association-dissociation curves for mIgG-Fab anti mIgG kinetic assay after 10 regeneration cycles. The data traces overlap closely, with low variability between calculated binding and affinity constants from cycle to cycle in the kinetic assay.

Table 4: The K_D and %CV for 10 cycles of regeneration for mIgG-Fab anti mIgG kinetic assay.

Kinetic Assay	**K_D(M)**	**k_a (1/Ms)**	**k_{dis} (1/s)**
Regen 1	9.22E-09	3.88E+04	3.58E-04
Regen 2	9.86E-09	3.83E+04	3.77E-04
Regen 3	1.04E-08	3.79E+04	3.93E-04
Regen 4	1.03E-08	3.84E+04	3.95E-04
Regen 5	1.05E-08	3.83E+04	4.01E-04
Regen 6	1.09E-08	3.80E+04	4.13E-04
Regen 7	1.06E-08	3.84E+04	4.08E-04
Regen 8	1.08E-08	3.87E+04	4.18E-04
Regen 9	1.06E-08	3.86E+04	4.08E-04
Regen 10	1.09E-08	3.90E+04	4.25E-04
Average (10 regenerations)	1.04E-08	3.84E+04	4.00E-04
%CV (10 regenerations)	5.0%	1%	5%

Figure 10: *Regeneration Assessment of AMC2 Biosensors for Quantitation Assays.*



Note: Overlay of binding curves for mIgG quantitation assay with mIgG concentrations in the range of 1-2000 µg/mL after 6 regeneration cycles using 10 mM Phosphoric acid.

Table 5: Calculated concentrations and %CV for 6 cycles of regeneration for mIgG quantitation assay.

Quantitation: Known Well Concentration (µg/mL)	Average Calculated Concertation (µg/mL) (6 regenerations)	%CV (6 regenerations)
2000	1850	10.5%
500	502.3	12.5%
50	50.03	4.4%
10	9.85	2.2%
5	5.22	2.4%
1	1.00	4.6%

Regeneration Tips

- Regenerate the Octet® AMC2 Biosensor surface after a kinetic or quantitation assay by dipping the biosensors into 10-100 mM Phosphoric acid for 60 seconds followed by neutralization in assay buffer for 5 seconds, then repeating these regeneration steps three to five times.
- Depending on the assay conditions or protein being captured, the regeneration buffer and/or conditions may require additional optimization.
- It is recommended to pre-condition the biosensors before the first assay cycle for most consistent results when incorporating regeneration. Biosensors are pre-conditioned by performing the regeneration procedure one time prior to the first loading step.
- It is important to ensure that the regeneration of biosensors for quantitation applications is complete. This is because the quantitation results are significantly dependent on surface capacity of the sensor. For example, a loss of 20% capacity over multiple regeneration cycles could affect precision of quantitation by 10–20%.

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