

Octet[®] AMC2 Biosensors

For Quantitation and Kinetic
Characterization of Murine
IgG Antibodies



Key Features

- Rapid quantitation and kinetic analysis of crude and purified murine IgG antibodies
- Increased biosensor loading capacity for improved kinetic characterization of smaller proteins
- Wide dynamic range for fast and accurate quantitation without dilution steps
- Efficient and cost-effective regeneration for biosensor re-use

Quick Facts

- High specificity to all mouse IgG (IgG1, IgG2a, IgG2b and IgG3) and rat IgG (IgG1, IgG2a, IgG2b and IgG2c) subclasses
- Dynamic range: 0.025 – 8,000 µg/mL
- Assay precision for quantitation: CV <10%

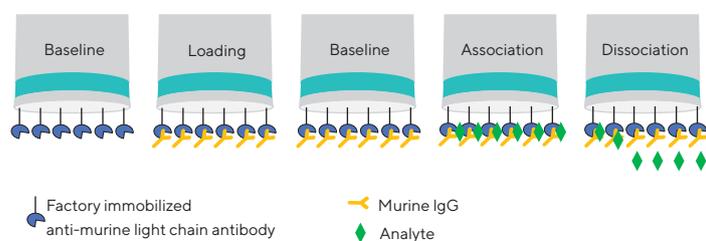
Overview

The Octet® AMC2 Biosensors are the next generation of anti-mouse IgG biosensors that offer improved performance and enable both quantitation and kinetic characterization of murine IgG antibodies on the Octet® Bio-Layer Interferometry (BLI) platform. These biosensors have high affinity to four mouse IgG (IgG1, IgG2a, IgG2b and IgG3) and rat IgG (IgG1, IgG2a, IgG2b and IgG2c) subclasses and can be used for analysis of mIgG in both crude and purified samples. The AMC2 biosensors feature up to 3-fold increased ligand binding capacity offering users enhanced kinetic characterization of smaller proteins and quantification of low antibody expression in mouse hybridoma samples. Also, the wide dynamic range of these biosensors allows users to accurately quantify mIgG proteins with concentrations up to 8 mg/mL without any dilution steps, thus making sample preparation fast and easy. 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 hybridoma screening, cell line development, process development and QC.

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. 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 small protein analytes. An example of the assay workflow utilizing the Octet® AMC2 Biosensors to characterize the interaction between an analyte and a mIgG is outlined in Figure 1.

A.



B.

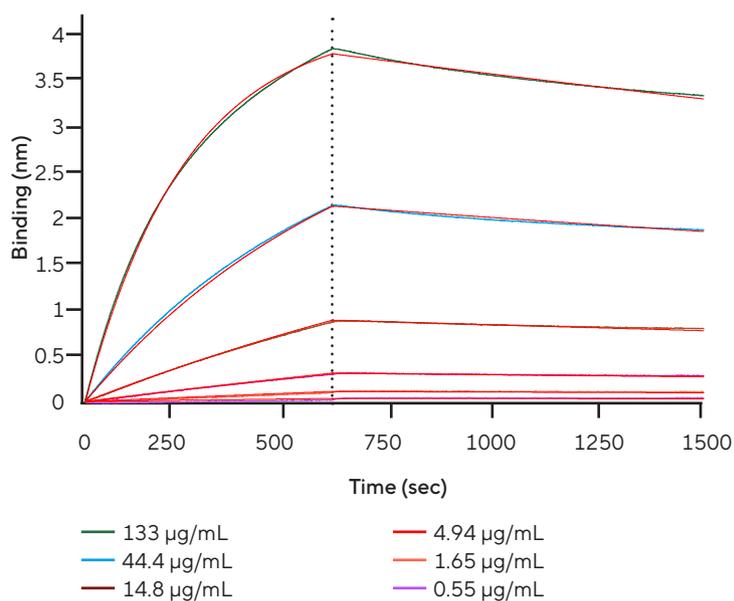


Figure 1: Kinetic characterization of the interaction between mouse IgG and an analyte using the Octet® AMC2 biosensors. (A) Kinetic assay workflow depicting the typical steps: 1 – equilibration (baseline), 2 – loading (capture) of a mIgG, 3 – baseline, 4 – association phase, 5 – dissociation phase. (B) Kinetic analysis of the interaction between a ligand mIgG1 (150 kDa) and an analyte Fab fragment, goat anti-mIgG (H+L) specific (50 kDa), on the Octet® RH16 BLI system.

Quantitation Assay Workflow

The Octet® AMC2 Biosensors can be used to quantitate both crude and purified mIgGs with concentrations in the range of 0.025 – 8,000 µg/mL. The antibody concentration is determined based on the rate of binding of mIgG to the biosensor surface and its comparison to the standard calibration curve constructed using identical mouse IgG protein sample with known concentrations. For standard sensitivity assay with mIgG concentration in the range of 0.5 – 8,000 µg/mL, it is recommended to use a shaking speed of 400 RPM with 1-min assay read time. However, for high-sensitivity assay with mIgG concentration in the range of 0.025–0.5 µg/mL, it is typically recommended to use 96-well plate as a sample plate and shaking speed of 1,000 RPM with 10-min assay read time. Determined IgG concentrations correlate well with results generated using orthogonal technologies, such as HPLC. An example of mIgG quantitation results generated using Octet® AMC2 Biosensors is shown in Figure 2.

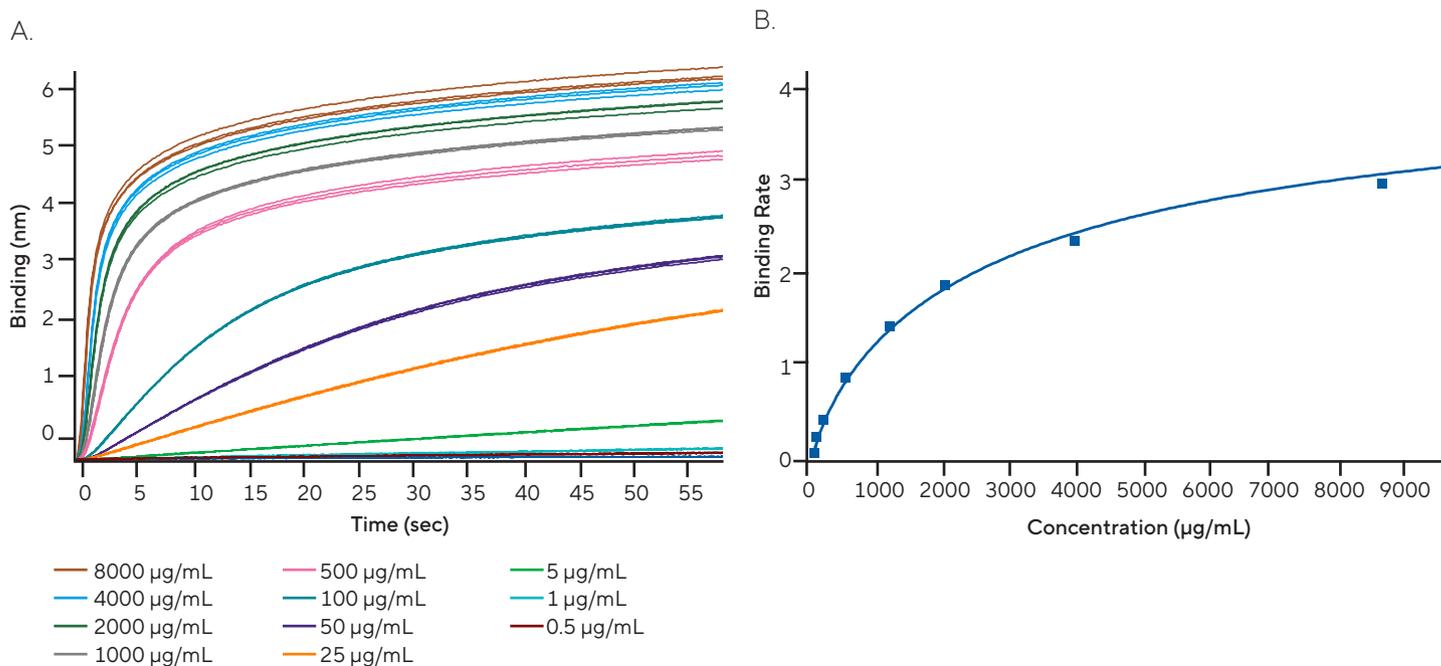


Figure 2: Quantitation of mIgG using the Octet® AMC2 Biosensors. (A) mIgG dose response for concentrations within the dynamic range of 0.5– 8,000 µg/mL. (B) mIgG standard calibration curve.

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%

Table 1: Calculated concentrations, %CV and %Recovery for mIgG (0.5–8,000 µg/mL) quantitation assay with triplicates.

Cost-Effective Regeneration

Octet® AMC2 Biosensors can be regenerated 8 - 10 times in both kinetic and quantitation assays using 10 - 100 mM Phosphoric acid solution in as little as 3 minutes. Regeneration allows for biosensor re-use and provides a cost-saving solution for generating replicate data for ligand-analyte pairs, or for analyzing large numbers of samples in sequence. Example of kinetics assay with 10 regeneration cycles is shown in Figure 3 and Table 2.

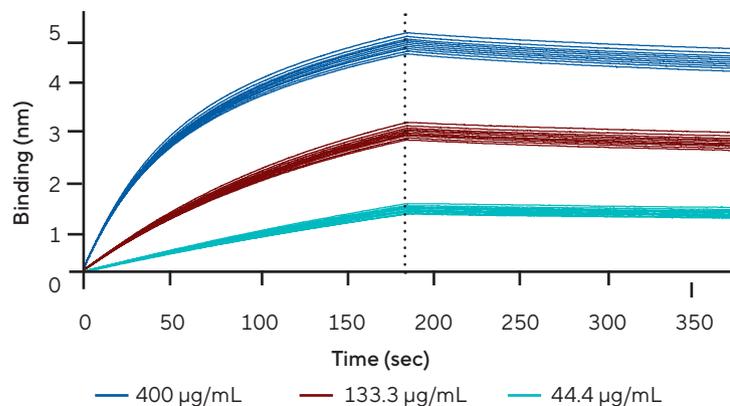


Figure 3: Overlay of association-dissociation curves for mIgG-Fab anti mIgG kinetic assay after 10 regeneration cycles using 10mM phosphoric acid. The data traces overlap closely, with low variability between calculated binding and affinity constants from cycle to cycle in the kinetic assay.

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%

Table 2: The K_D and %CV for 10 cycles of regeneration for mIgG-Fab anti mIgG kinetic assay.

Ordering Information

Description	UOM	Cat. No.
One tray of Octet® AMC2 Biosensors	Tray	18-5163
Five trays of Octet® AMC2 Biosensors	Pack	18-5164
Twenty trays of Octet® AMC2 Biosensors	Case	18-5165
Octet® Sample Diluent Buffer, 50 mL	Each	18-1104
Octet® Kinetics Buffer 10X (10X KB), 50 mL	Each	18-1105

Germany

Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA

Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 888 OCTET 75
Or +1 650 322 1360

 For further information, visit
www.sartorius.com