SARTORIUS

Product Datasheet

Octet® AHC2 Biosensors

For Quantitation and Kinetic Characterization of Human Fc-Region Containing Proteins

Key Features

- Rapid quantitation and kinetic analysis of crude and purified human Fc-region containing proteins.
- Improved biosensor surface ligand loading capacity for enhanced kinetics assays sensitivity and increased dynamic range for quantitation assays.
- Efficient and cost-effective biosensor regeneration for multiple kinetics or quantitation measurements.

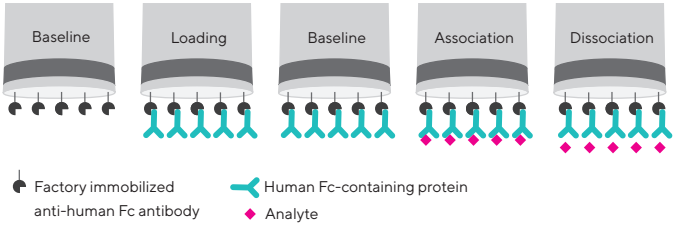
Overview

The Octet® AHC2 Biosensors are the next generation of anti-human IgG Fc capture biosensors that offer significantly improved performance as well as increased versatility by enabling both quantitation and kinetic characterization of human IgG antibodies and IgG-derived Fc-fusion proteins. Furthermore, the Octet® AHC2 Biosensors can be efficiently and cost-effectively regenerated 10-20 times for both kinetics and quantitation applications, which makes them an extremely useful solution for a wide range of high-throughput applications, including lead identification and optimization, cell line development, process development and QC.

Kinetic Assay Workflow

The Octet® AHC2 Biosensors are pre-immobilized with a new anti-human Fc-specific antibody, which enables capturing of human IgG and human Fc-region containing proteins directly from a crude or purified matrix. They provide up to 2-fold increased binding capacity for human IgG and Fc-region containing proteins when compared to the previous generation of the AHC biosensors, thus making them particularly suitable for high sensitivity kinetic assays such as the characterization of smaller proteins. An example assay workflow utilizing the Octet® AHC2 Biosensors to characterize the interaction between an analyte and a human IgG is outlined in Figure 1.

A.



B.

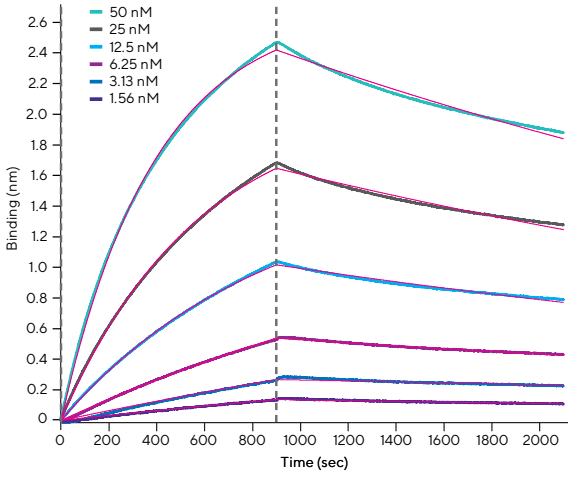


Figure 1: Kinetic characterization of the interaction between human IgG and an analyte using the Octet® AHC2 Biosensors. (A) Kinetic assay workflow depicting the typical steps: 1. equilibration (baseline), 2. loading (capture) of a hIgG, 3. baseline, 4. association kinetics, 5. dissociation kinetics. (B) Kinetic analysis of the interaction between ligand hIgG and an analyte Fab fragment, goat anti-hIgG (H+L) specific (50 kDa) on the Octet® platform. The association and dissociation traces were fit to a 1:1 binding model. The kinetic results are reported in Table 1.

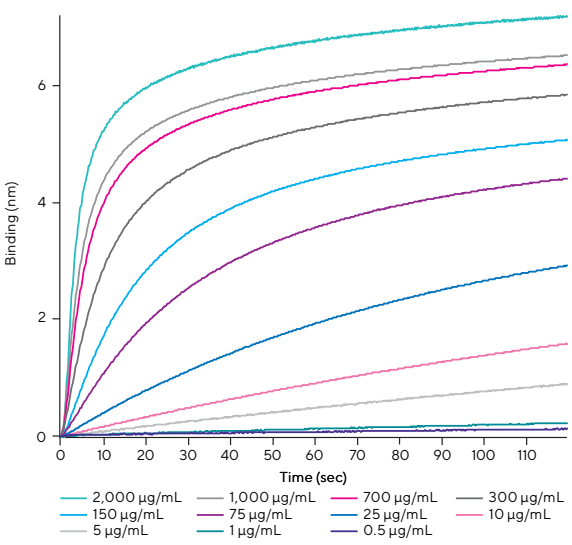
Table 1: Kinetic results for the interaction between ligand hIgG and an analyte Fab fragment, goat anti-hIgG (H+L) specific (50 kDa) using Octet® AHC2 Biosensors for the data shown in Figure 1B.

k_{on} (1/Ms)	k_{off} (1/s)	K_D (M)
4.82E+04	2.33E-04	4.84E-09

Quantitation Assay Workflow

The Octet® AHC2 Biosensors determine antibody concentration based on the rate of binding of the Fc-region of the protein to the biosensor surface. More specifically, the quantitation is achieved by comparison of the binding rate to that of a standard calibration curve constructed from identical human Fc-containing protein samples with known concentrations. The Octet® AHC2 Biosensors have high specificity towards all four hIgG subclasses and can be used to quantitate both crude and purified hIgGs with concentrations in the range of 0.1–2,000 µg/mL depending on the assay conditions. Determined IgG concentrations correlate well with results generated using orthogonal technologies, such as HPLC. An example of hIgG quantitation results generated using Octet® AHC2 Biosensors is shown in Figure 2.

A.



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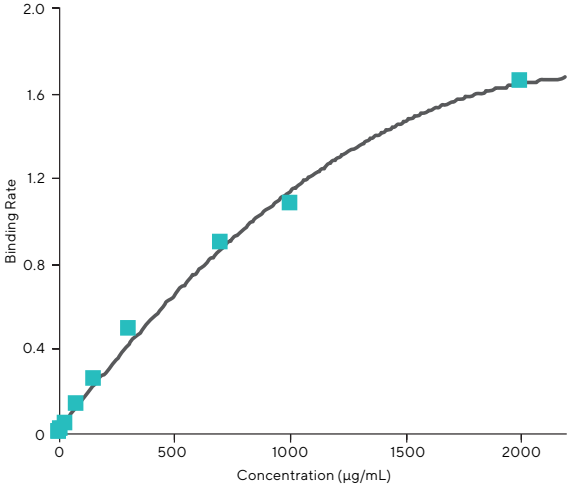

Figure 2: Quantitation of hIgG using the Octet® AHC2 Biosensors. (A) hIgG dose response for concentrations within the dynamic range of 0.5–2,000 µg/mL. (B) hIgG standard calibration curve calculated using 5PL (weighted Y2) fitting model.

Cost-Effective Regeneration

The Octet® AHC2 Biosensors can be regenerated 10–20 cycles in both kinetic and quantitation assays via a standard low pH-protocol in as little as 2 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 quantitation assay with 10 regeneration cycles is shown in Figure 3 and Table 2.

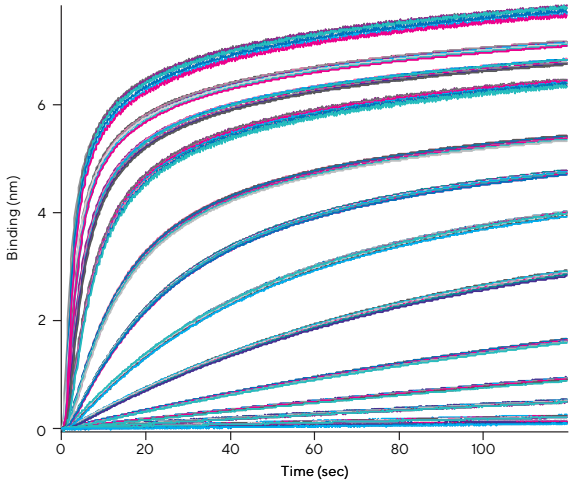

Figure 3: Overlay of binding curves for hIgG quantitation assay after 10 regeneration cycles using 10 mM Glycine, pH 1.7.

Table 2: Calculated concentrations and %CV for 10 cycles of regeneration for hIgG quantitation assay.

Well Concentration, µg/mL	Average Calculated Concertation after 10 regenerations	%CV after 10 regenerations
2000	2052.29	8%
1000	975.92	5%
500	508.66	3%
200	199.03	2%
100	100.36	1%
50	51.16	1%
25	24.60	1%
10	9.92	1%
5	4.94	2%
2.5	2.61	5%
1	0.98	4%
0.5	0.50	6%

Range of Applications

The Octet® AHC2 Biosensors offer a flexible platform for a broad range of applications, from profiling the kinetics (k_{on}, k_{off}, and K_D) or performing epitope binning between human Fc-containing proteins and their analytes, to quantitation of human Fc-containing proteins in both purified and crude protein samples (e.g. supernatants and sera).

Quick Facts

Applications	Both quantitation and kinetic characterization of human IgG antibodies and and IgG-derived Fc-fusion proteins
Dynamic range for quantitation applications	0.5–2,000 µg/mL at 400 rpm
Limit of detection for quantitation	0.1 µg/mL at 1,000 rpm
Precision for quantitation	<10% CV

Ordering Information

Part No.	UOM	Description
18-5142	Tray	One tray of 96 Octet® AHC2 Biosensors
18-5143	Pack	Five trays of 96 Octet® AHC2 Biosensors
18-5144	Case	Twenty trays of 96 Octet® AHC2 Biosensors

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