

Anti-human IgG Fc Capture (AHC) Biosensors

Immobilization and Subsequent Kinetic Characterization of Human IgGs and other Fc-Containing Proteins

Key Features

- Capture-based immobilization of human IgGs or other Fc-containing proteins
- Biosensor can be regenerated and reused
- Designed for use in buffer or complex media

The Anti-Human IgG Fc Capture (AHC) biosensor is designed for kinetic characterization of macromolecular interactions between proteins containing an Fc region and a target analyte. This biosensor enables the immobilization of human IgG or other protein containing a human Fc region using a high-affinity anti-human Fc-specific antibody. The biosensor surface has been designed to provide stable interactions and can be regenerated, making it particularly suitable for high-throughput kinetics applications. AHC biosensors can be used with many sample types, including purified proteins and cell culture supernatants.

QUICK FACTS

- **Method of Immobilization:** Capture of human Fc-containing proteins through a high-affinity anti-human Fc antibody
- **Baseline Stability:** 60 minutes
- **Number of Acceptable Regeneration Cycles:** 10

CAPTURING HUMAN IGG FROM COMPLEX MIXTURES

AHC biosensors were designed to immobilize hlgG from either buffer or cell culture media. Directly pulling hlgG from cell culture media and performing kinetic analyses is a much-simpler workflow than purification and biotinylation. Figure 1 illustrates the capture of hlgG using AHC biosensors. Biosensors A and B show the capture of hlgG from buffer, while biosensors C and D show hlgG capture from CD-CHO media containing fetal bovine serum (FBS).

REGENERATION AND REUSE OF THE BIOSENSOR

The AHC biosensors can be regenerated through exposure to low pH. This restores the biosensor's anti-hlgG surface, allowing reuse of the biosensor to perform additional analyses. Regeneration of the surface requires brief exposure to low pH followed by exposure to a neutral solution. Regeneration typically takes one to two minutes, but may vary depending on the hlgG. Figure 2 illustrates an assay in which the biosensors were regenerated four times. Each biosensor delivered very consistent data throughout all four cycles of kinetic analysis.

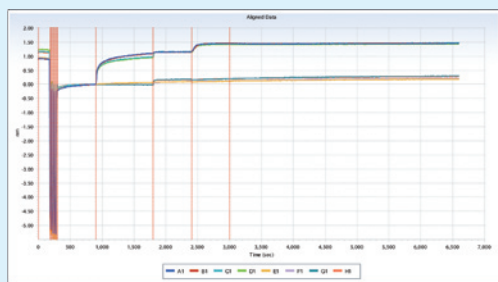


FIGURE 1: Real-time assay chart showing surface conditioning, hlgG capture, and subsequent analyte binding.

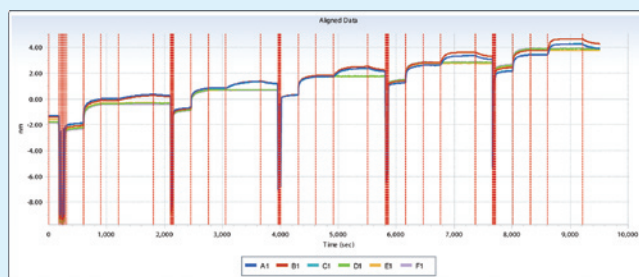


FIGURE 2: Capture of a hlgG and subsequent kinetic analysis of a protein analyte (Biosensors A-C). Biosensors D-F were used for baseline reference. The same biosensors were used five times in total, and were regenerated four times.

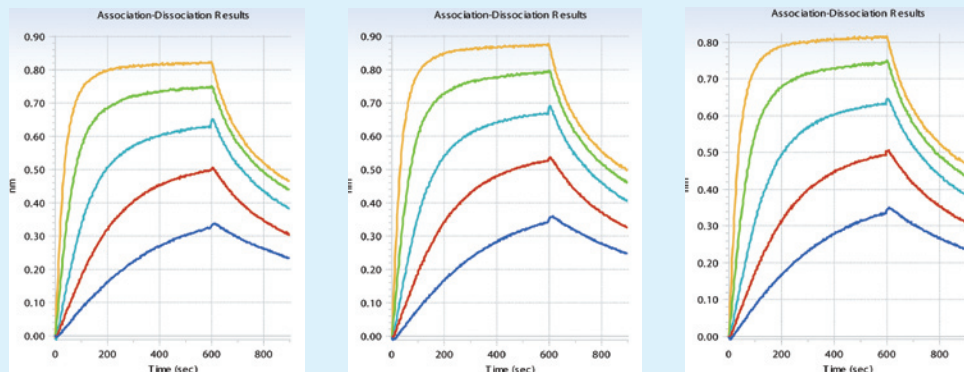


FIGURE 3: Analyte association and dissociation data for all analyte concentrations using biosensors A-C.

	$k_{on}(1/Ms)$	$k_{dis}(1/s)$	$K_D(M)$
hIgG captured from buffer	7.34E+05	8.69E-05	1.21E-10
hIgG captured from media	7.47E+05	9.45E-05	1.27E-10

TABLE 1: Kinetic constants from the hIgG/ analyte interaction are equivalent irrespective of whether the hIgG was captured from buffer or media containing FBS.

APPLICATIONS

The AHC biosensors provide a flexible platform for thorough kinetic profiling of an hIgG-analyte pair, for screening interaction kinetics of hIgG antibodies with relevant analytes, or for performing epitope mapping studies of the captured hIgG. For screening applications, assays can easily be designed to facilitate screening up to 72 hIgGs against a single analyte in one experiment. If incorporating regeneration, the AHC biosensors can be used to screen up to 56 hIgGs against a single analyte in one experiment. The capture surface can be regenerated and reused up to 10 times, making the AHC biosensor a cost-effective, easy-to-use solution for fast and accurate hIgG kinetic screening.

TYPICAL ASSAY PARAMETERS

- **Sample volume:** 200 μ L/well (post-dilution)
- **Hydration solution volume:** 200 μ L/well
- **Flow rate:** 1000 rpm
- **Biosensor hydration and sample plate equilibration:** 10 minutes

For more information about ForteBio's Octet platform for label-free, real-time detection of biomolecular interactions, applications, and services, visit www.fortebio.com or contact us directly.

ORDERING INFORMATION

Part No.	UOM	Description
18-5060	Tray	Tray of 96 biosensors, includes 1 bottle 10X kinetics buffer
18-5063	Pack	Five trays of 96 biosensors, includes 1 bottle 10X kinetics buffer
18-5064	Case	Twenty trays of 96 biosensors, includes 1 bottle 10X kinetics buffer

Materials Required But Not Provided

Microplates: Two 96-well, black, flat bottom, polypropylene microplates (Greiner Bio-one Cat. No. 655209).

NOTE: Autogain calibration occurs during biosensor pick-up (in the hydration plate), so it is important to use a black flat bottom plate for hydration to provide a dark, non-reflective environment similar to that in which the interaction will be measured.

Human IgG or other human Fc-containing protein(s) to be immobilized: The human IgG can be pure or part of a complex mixture such as cell culture supernatant.

Conditioning/Regeneration Solution: 10 mM Glycine, pH 1.7.

Assay Buffer: 1X Kinetics Buffer recommended. Other buffers may be used. For best results, we recommend keeping the buffer consistent throughout hydration and all assay steps.