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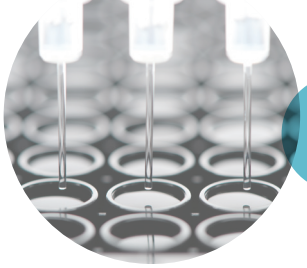

Anti-human IgG Fc Capture (AHC) Biosensors

For kinetic characterization of interactions between analytes and human Fc-containing proteins

Key features

- Capture-based immobilization of human Fc-containing proteins
- Regenerable and cost-effective format
- Compatible with crude samples and complex media

The Dip and Read™ Anti-Human IgG Fc Capture (AHC) biosensors enable kinetic characterization of macromolecular interactions between human Fc-containing proteins and target analytes. Immobilization of human Fc-containing proteins is achieved through a factory immobilized anti-human Fc-specific antibody whose high-affinity for the human Fc domain provides the stable baseline required for demanding kinetics applications. Cost-effective regeneration of the biosensors and the ability to directly immobilize human Fc-containing proteins from crude matrices make the AHC Biosensor extremely useful in high-throughput applications.

Streamlined workflow

The traditional workflow for measuring k_{on} and k_{off} between a human Fc-containing protein and an analyte requires labor-intensive steps non-conducive to high-throughput screening: purification of the Fc-containing protein, biotinylation of the purified protein and, finally, immobilization on a Streptavidin Biosensor. AHC biosensors streamline this workflow by enabling immobilization of human Fc-containing proteins upon the biosensor directly from a crude or purified matrix. No purification or biotinylation steps are required, and thereby high-throughput screening methods are facilitated. Figure 1 illustrates the efficient workflow of characterizing the interaction between an analyte and a human IgG that is captured from a crude matrix. After a biosensors A and B capture purified hIgG from a buffer matrix (Step 2), establish a baseline by equilibration in buffer (Step 3) and then detect association (Step 4) and dissociation of an analyte (Step 5). Biosensors C and D achieve equivalent results by capturing the hIgG from CD-CHO media containing fetal bovine serum (FBS), representative of a crude protein sample.

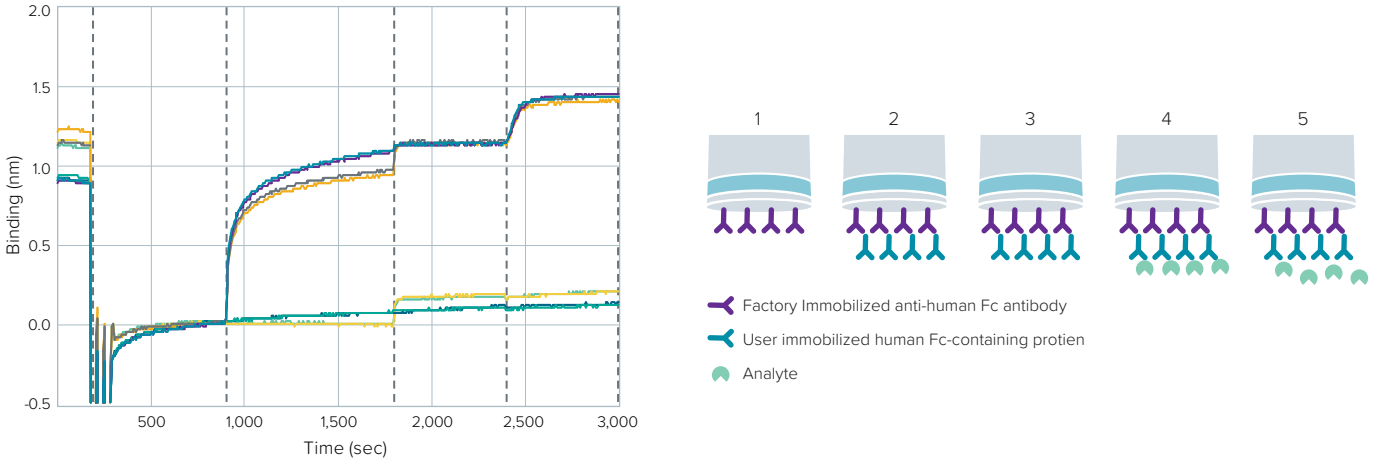

Figure 1: Kinetic characterization of the interaction between hIgG and an analyte using AHC biosensors within a five-Step assay. Step 1 – baseline, Step 2 –loading of hIgG, Step 3 – baseline, Step 4- analyte-hIgG association kinetics, Step 5 – analyte-hIgG dissociation kinetics. All measurements performed in triplicate. Biosensors A–C delivered reproducible values for loading, association and dissociation steps. Reference biosensors (D–F) delivered reproducible low background values, indicating that binding of hIgG and the analyte are protein-specific interactions.

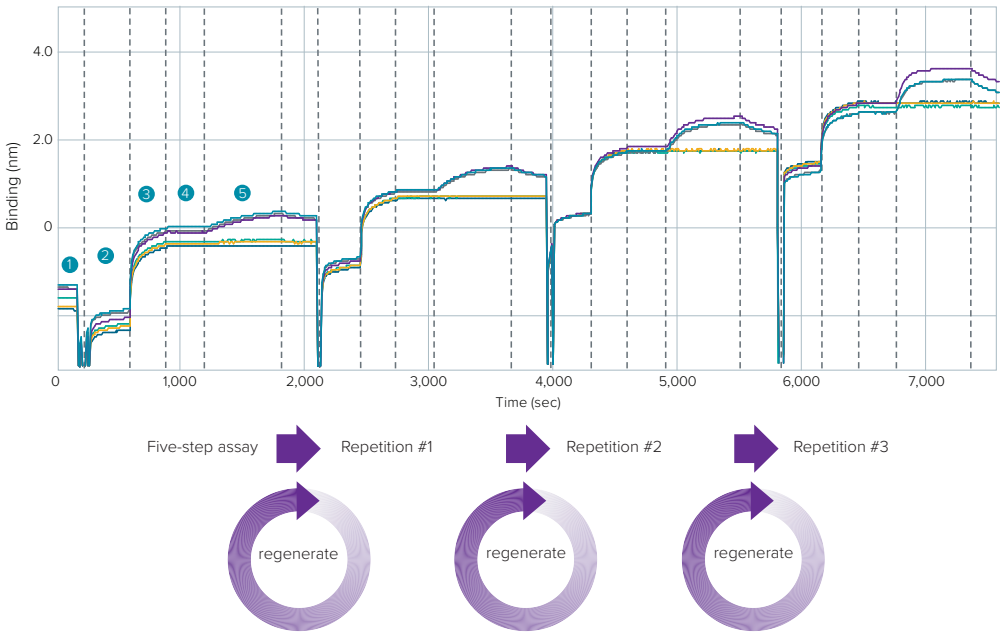

Figure 2: Repetition of a five-step kinetic assay with regenerated AHC biosensors. Step 1 – baseline, Step 2 – hIgG loading, Step 3 – baseline, Step 4 – analyte-hIgG association kinetics, Step 5 – dissociation kinetics. All kinetic measurements (biosensors A–C) and baselines (biosensors D–F) were performed in triplicate. Biosensors were used four times in total with three regeneration cycles.

Cost-effective regeneration

AHC biosensors can be regenerated up to 10 times via a standard low-pH protocol in as little as two minutes. Regeneration dissociates the human-Fc-containing protein from the anti-human Fc antibody, allowing additional analyses and provides a cost-effective format for analyzing large sample libraries. Figure 2 illustrates three-fold regeneration of AHC biosensors with minimal loss in capacity in a five-step assay. After establishing an initial baseline (Step 1), loading a human Fc-containing protein (Step 2) and a second baseline (Step 3), the association kinetics (Step 4) and dissocation kinetics (Step 5) of an analyte are recorded. Each five-step assay was followed by a brief regeneration protocol. Comparison of the loading, association and dissociation steps for each analysis demonstrates robust and reproducible data collection.

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

Flexible applications

AHC biosensors provide a flexible platform for profiling the kinetics between human Fc-containing proteins and their analytes. During antibody development, AHC biosensors enable rapid epitope mapping and binning studies of captured human Fc-containing proteins, such as hIgGs. In a single experiment, 72 hIgGs can be screened against a single analyte in as little as one hour using an Octet® RED instrument. Alternatively, using an Octet RED384 instrument increases throughput to 352 samples in as little as one hour.

Ordering Information

Part No.	UOM	Description
18-5060	Tray	One tray of 96 Anti-Human Fc Capture (AHC) biosensors
18-5063	Pack	Five trays of 96 Anti-Human Fc Capture (AHC) biosensors
18-5064	Case	Twenty trays of 96 Anti-Human Fc Capture (AHC) biosensors

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