

Octet® 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 Octet® 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 an 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. The Octet® 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 human IgG (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.

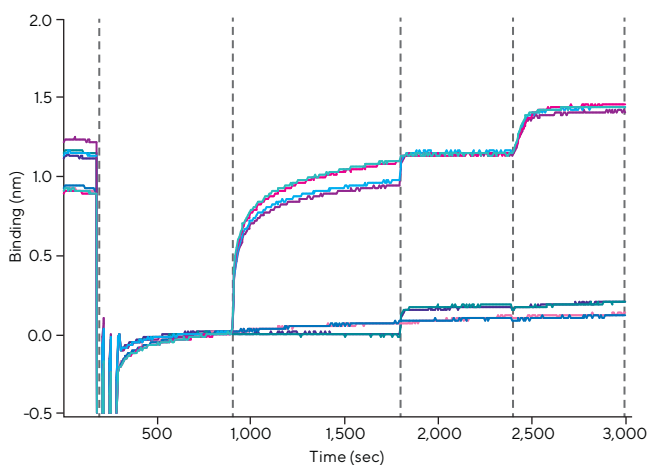
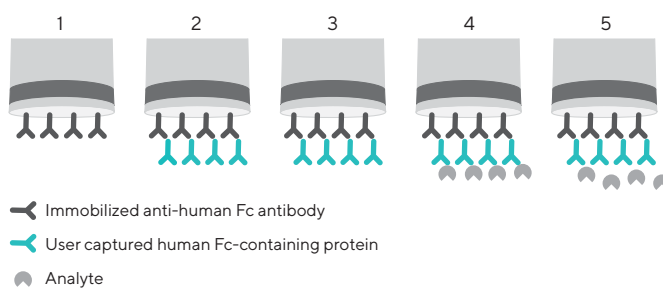


Figure 1: Kinetic characterization of the interaction between hIgG and an analyte using the Octet® AHC biosensors within a five-step assay. Step 1 – baseline, Step 2 – capture of hIgG, Step 3 – baseline, Step 4 – analyte-hIgG association kinetics, Step 5 – analyte-hIgG dissociation kinetics. All measurements 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.

Cost-Effective Regeneration

The Octet® 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 dissociation 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.



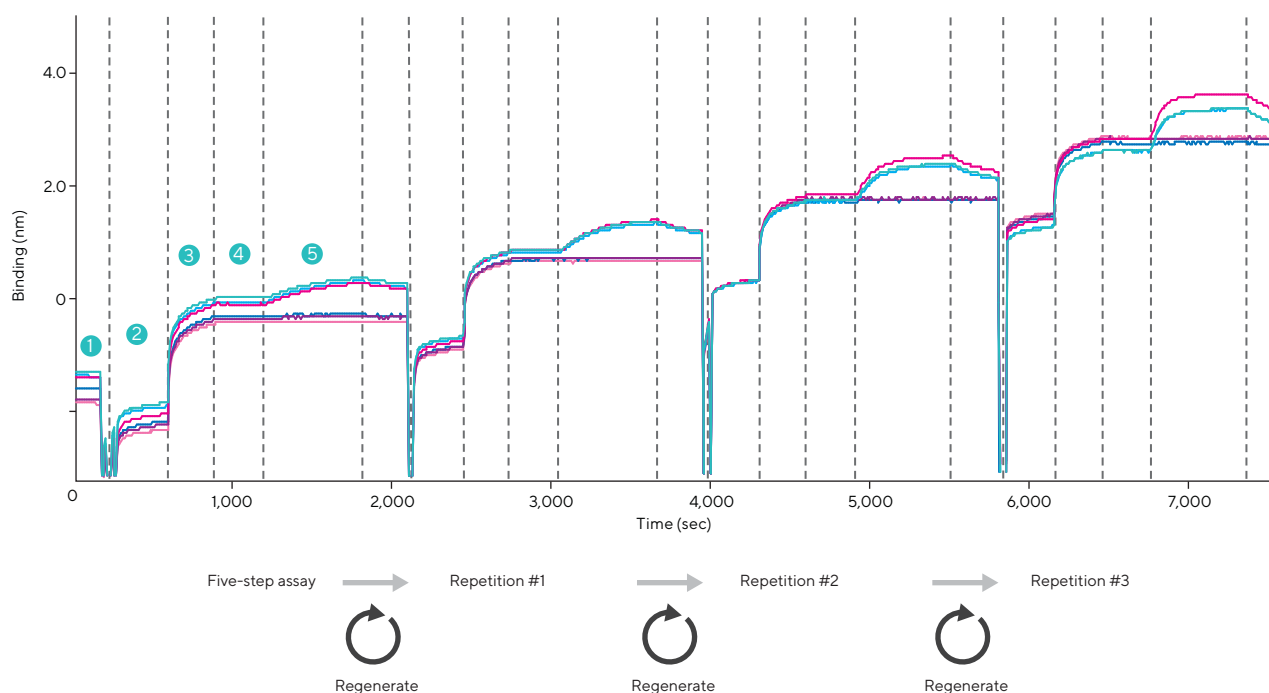


Figure 2: Repetition of a five-step kinetic assay with regenerated AHC biosensors. Step 1 – baseline, Step 2 – hlgG loading, Step 3 – baseline, Step 4 – analyte-hlgG association kinetics, Step 5 – dissociation kinetics. All kinetic measurements (biosensors A–C) and baselines (biosensors D–F) were performed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

Flexible Applications

The Octet® 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 binding studies of captured human Fc-containing proteins, such as hlgGs. In a single experiment, 72 hlgGs can be screened against a single analyte in as little as one hour using an Octet® R8 instrument. Alternatively, using an Octet® RH16 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 Octet® AHC Biosensors coated with anti-human IgG for the immobilization of human IgG or other proteins containing the human Fc region for kinetic analysis. |
| 18-5063 | Pack | Five trays of 96 Octet® AHC Biosensors coated with anti-human IgG for the immobilization of human IgG or other proteins containing the human Fc region for kinetic analysis. |
| 18-5064 | Case | Twenty trays of 96 Octet® AHC Biosensors coated with anti-human IgG for the immobilization of human IgG or other proteins containing the human Fc region for kinetic analysis. |

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