

Anti-Mouse IgG Fc Capture (AMC) Biosensors

Immobilization and subsequent kinetic characterization of mouse IgGs and other Fc-containing proteins

Key features

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

Overview

Dip and Read™ Anti-Mouse IgG Fc Capture (AMC) biosensors enable kinetic characterization of macromolecular interactions between mouse Fc-containing proteins and target analytes. Immobilization of mouse Fc-containing proteins is achieved through a factory immobilized anti-mouse Fc-specific antibody whose high-affinity for the mouse Fc domain provides the stable baseline required for demanding kinetics applications. Cost-effective regeneration of the biosensors and the ability to directly immobilize mouse Fc-containing proteins from crude matrices make the AMC Biosensor extremely useful in high-throughput applications. Subtypes IgG1, IgG2a and IgG2b are recommended for use with AMC biosensors; IgG3 should be evaluated on a case-by-case basis.

Principle

The Anti-Mouse IgG Capture (AMC) biosensors are pre-immobilized with a high affinity antibody against the Fc portion of mouse IgG. This antibody can capture and immobilize mouse IgG (mIgG) or other Fc-containing ligands to produce a stable surface suitable for interaction analysis. The capture surface is particularly suited for immobilizing mIgG from cell culture supernatants or other complex mixtures where biotinylation is not an option.

Streamlined workflow

The traditional workflow for measuring k_{on} and k_{dis} between a mouse Fc-containing protein and an analyte requires labor intensive steps that are both inconvenient and non-conducive to high-throughput screening: purification of the Fc-containing protein, biotinylation of the purified protein and, finally, immobilization of the ligand on a Streptavidin Biosensor. Off-the-shelf AMC biosensors streamline this workflow by enabling immobilization of mouse 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).

AMC biosensors can be regenerated up to 10 times via a standard low-pH protocol in as little as two minutes for select applications such as acquisition of replicate data (same ligand/analyte pair) and “bucket”-based screening applications. Regeneration dissociates the mouse Fc-containing protein from the factory-immobilized anti-mouse Fc antibody, allowing additional analyses. For best final affinity and kinetic determination results, using a new AMC Biosensor for each unique capture ligand is recommended.



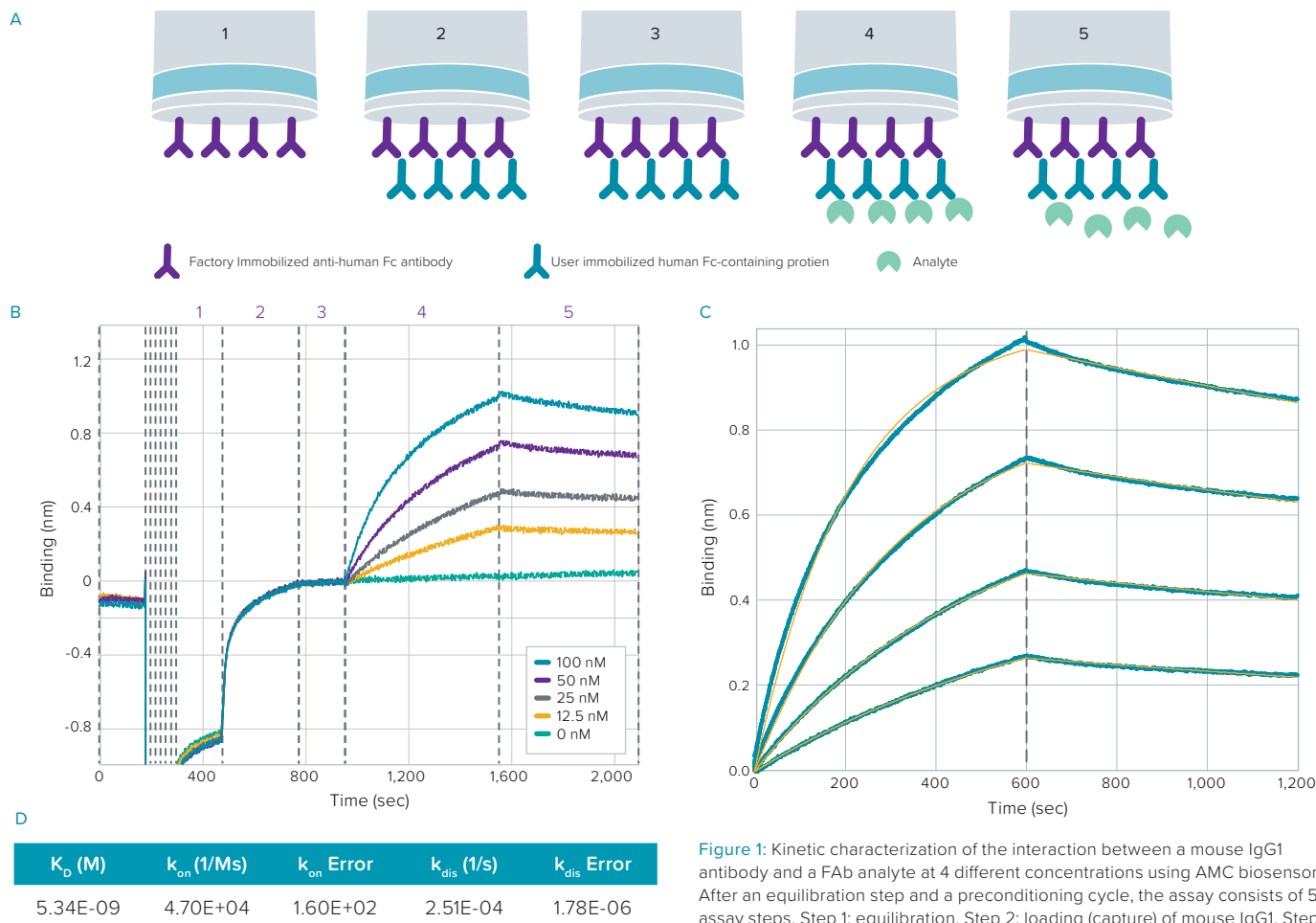


Figure 1: Kinetic characterization of the interaction between a mouse IgG1 antibody and a FAb analyte at 4 different concentrations using AMC biosensors. After an equilibration step and a preconditioning cycle, the assay consists of 5 assay steps. Step 1: equilibration, Step 2: loading (capture) of mouse IgG1, Step 3: baseline, Step 4: association kinetics, Step 5: dissociation kinetics. The raw data for a full assay is shown in Fig 1B. After data processing (including reference subtraction using the 0 nM trace), the association and dissociation traces were fit to a 1:1 binding model (1C, red lines). Values of K_D , k_{on} and k_{dis} were extracted from the curve fitting analysis (1D).

Flexible applications

AMC biosensors provide a flexible platform for evaluating the kinetics between mouse Fc-containing proteins and their analytes.

- Complete kinetic analysis (k_{on} , k_{dis} and K_D) between mouse Fc containing proteins and target analytes
- Off-rate ranking of hybridoma and stable cell-line supernatants
- Epitope binning/mapping (from crude or purified samples)
- Tracking product integrity by measuring k_{on} , k_{dis} and K_D during:
 - Upstream fermentation
 - Downstream harvest and purification
 - Post-derivatization (pegylation)
 - Formulation development

Ordering information

| Part No. | UOM | Description |
|----------|------|---|
| 18-5088 | Tray | One tray of 96 Anti-Mouse IgG Fc Capture (AMC) biosensors |
| 18-5089 | Pack | Five trays of 96 Anti-Mouse IgG Fc Capture (AMC) biosensors |
| 18-5090 | Case | Twenty trays of 96 Anti-Mouse IgG Fc Capture (AMC) biosensors |

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