



Crude Samples Analysis

Screening and Characterization of Hybridoma Supernatants

OBJECTIVE

Primarily, to screen mouse IgG antibodies from crude samples, such as hybridoma supernatants, for dissociation rates of bound antigen. In addition, to determine detailed kinetic rate constants and affinity between the antigen and an antibody selected in the screen.

CONCLUSIONS

- A real time, label-free method to capture mouse IgG from hybridoma supernatants has been developed using the Attana A100[®] C-Fast system. The same approach can be replicated for capture of human IgGs.
- Kinetic information about the interactions, association (k_{on}) and dissociation (k_{off}) rate constants are derived to calculate the affinity (K_D) between the molecules.
- Facilitation for rapid screening of up to 192 antibody-antigen pairs directly from crude samples, such as hybridoma supernatants, without the necessity to purify or label the antibody is achieved.
- The method is time saving as generic immobilization and regeneration conditions have been established and no prior purification of the antibody is necessary.

BACKGROUND

Purification, and sometimes labeling, of antibodies before characterization of the antibody-antigen interactions can be very time consuming and associated with issues such as optimization and yield. The Attana A100 C-Fast system together with the Attana IgG capture kits can reduce these problems. Here we demonstrate an anti-mouse IgG surface with the ability to capture antibodies from crude materials, followed by a detailed study of the kinetics for the interactions between the antibody and its antigen.

ATTANA A100 C-FAST BIOSENSOR

The Attana A100 C-Fast biosensor utilizes the Quartz Crystal Microbalance (QCM) technique for real time, label-free measurements of molecular interactions. When molecules are added to, or removed from the sensor surface, the change in the resonance frequency corresponds to the change in mass on the surface. By immobilizing a target molecule to the sensor surface, and flowing an interacting molecule over the surface, the interaction can be studied in real time. The real-time information can provide kinetic, affinity and specificity data on the interaction.

METHOD

A mouse IgG capture molecule was immobilized on an Attana Carboxyl Sensor Chip surface in 1xHBST (10mM HEPES buffered saline with 0.005% Tween[®] 20 at 25 μ l/min, 22°C) according to instructions provided in the Mouse-IgG

Capture Kit. Mouse IgG antibodies were then captured from hybridoma supernatants, and their respective interaction properties, with the antigen, were studied in real time. An antigen concentration of 5 μ g/ml was used to determine the dissociation rate constants (the off-rates) while concentrations between 1.25 and 20 μ g/ml were used for detailed kinetic characterization. The surface was regenerated after each interaction cycle, restoring the initial mouse IgG capture capacity.

Data were collected using the Attester[™] 3.1 software and, subsequently, processed in the Attester[™] Evaluation 3.1. The embedded off-rate screening tool in Attester Evaluation was used to identify the antibody with the lowest dissociation rate constant for the antigen. To calculate and obtain detailed kinetic data, Attester Evaluation and Clamp XP^{*} were used.

RESULTS

Dissociation rate constants for 11 antibodies, from mouse IgG producing hybridoma cultures, were screened against an antigen. Firstly, the mouse IgG binding molecule was used to capture IgG from the respective supernatants. After which, the antigen was injected over the surface and the interaction with the captured antibody was monitored. The Attester Evaluation off-rate screening tool was used to generate a list, highlighting the most stable interactions (low dissociation rate constants). Refer to **Fig. 1** for the sensorgram and the collected data.

Balancing **Power** and **Simplicity**
in Molecular Interaction Studies



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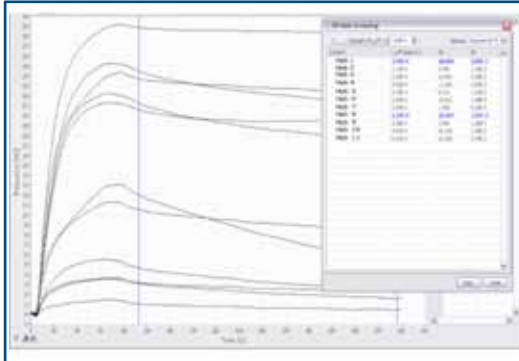


Figure 1: Compilation of antigen interaction curves for 11 different antibodies. For each interaction cycle, one specific antibody – containing hybridoma supernatant – was injected for capturing of the antibodies on the surface, followed by injection of the antigen. Also shown in the figure is the Attester Evaluation off-rate screening tool. The table presents the 11 determined dissociation constants. The threshold function is used to highlight the antibodies with a dissociation rate constant lower than a certain value. As shown, dissociation rate constants lower than 1.8×10^{-4} are highlighted in blue.

Content	k _{off}
Hybridoma 1	3.50E-5
Hybridoma 2	1.17E-3
Hybridoma 3	2.06E-4
Hybridoma 4	7.92E-4
Hybridoma 5	2.43E-3
Hybridoma 6	2.97E-3
Hybridoma 7	2.94E-3
Hybridoma 8	1.35E-4
Hybridoma 9	2.35E-3
Hybridoma 10	5.07E-4
Hybridoma 11	6.61E-4

Attana Materials Used	Item Code
Attana Carboxyl Sensor Chip	3616-3033 (pack of 3) 3616-3103 (pack of 10)
Mouse-IgG Capture Kit: Fc-specific	3518-3001
Amine Coupling Kit	3501-3001
HBS-T 10X (250 ml)	3506-3001
C-Fast: 3.1	3420-3001
Attester™: 3.1	3410-3001
Attester™ Evaluation: 3.1	3430-3001

The hybridoma with the lowest off-rate was chosen for detailed kinetic characterization of the antibody-antigen interactions. The same capturing surface, as used for the off-rate screening, was used in this step. While keeping the antibody capturing level constant and varying the antigen concentration, detailed kinetic rate constants were established by analyzing the data using a global curve fit algorithm (i.e. to derive k_{on} , k_{off} and K_D of the interaction, Fig. 2).

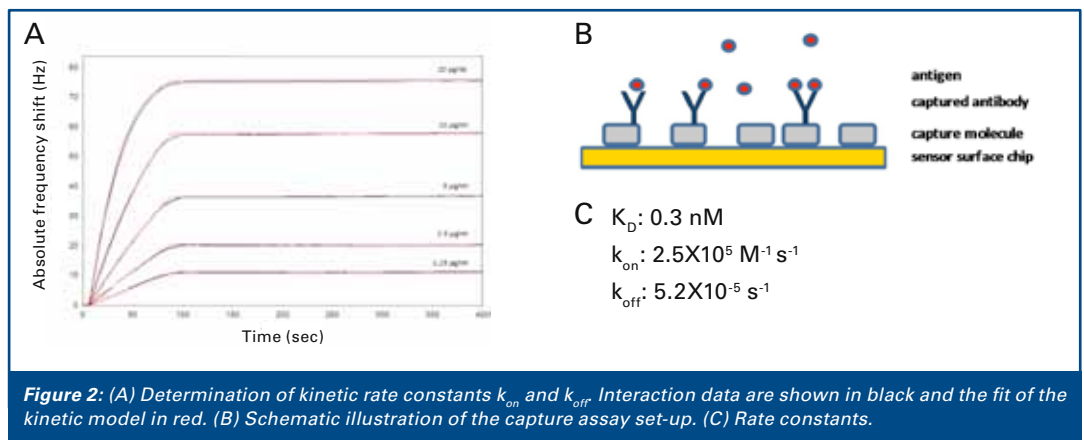


Figure 2: (A) Determination of kinetic rate constants k_{on} and k_{off} . Interaction data are shown in black and the fit of the kinetic model in red. (B) Schematic illustration of the capture assay set-up. (C) Rate constants.

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