



Crude Samples Analysis

Direct off-rate screening in Supernatants and Lysates

OBJECTIVE

To determine off-rates of antibodies in mouse hybridoma supernatants and scaffold proteins in bacterial lysates using the Attana 200 system and Low Non-specific Binding (LNB) surfaces.

CONCLUSIONS

- A real time, label-free, direct method to screen antibodies in hybridoma supernatants and scaffold proteins in bacterial lysates has been developed, using the dual-channel Attana 200 system and LNB surfaces.
- By using this method, dissociation rate constants (k_{off}) and ranking information can easily be obtained. Allowing for screening of up to 192 samples without attention.
- Attana's proprietary low non-specific binding surfaces extend the possibilities for screening in crude samples, such as supernatants, serums and lysates, without the need for using capture set-up's
- The method can be used for clone selection, vaccine screening, biomarker discovery, biomanufacturing and performance studies in biologically relevant environments.
- The assay, together with Attana's proprietary technologies, provides time, labour and cost effective methods for analysis in crude samples and in biologically more relevant contexts.

BACKGROUND

When working with crude samples, such as supernatants, lysates or serum, these often have to be purified in order for interaction constants to be determined. This is time-consuming, labour-intensive and results in loss-of-yield. Screening of hybridoma supernatants using a capture set-up has previously been shown. However, this consumes a lot of antigen, generates long assay times and carries the risk that matrix components are reactive against the capturing molecule and not the analyte. The ability to analyse crude samples in a direct set-up would thus provide the benefits of saving time, labour, cost and avoid known issues with the capture approach. However, this put high demands on low non-specific binding.

Here we demonstrate the ability of the dual-channel Attana 200 system and Attana's proprietary LNB carboxyl surfaces, to screen unpurified *E. coli* lysates and serum containing hybridoma supernatants directly against an antigen immobilised on the sensor surface.

ATTANA 200 BIOSENSOR

The Attana 200 biosensor utilizes the Quartz Crystal Microbalance (QCM) technique for real-time, label free measurement of molecular interactions. When molecules are added to, or removed from the surface, the change in the resonance frequency corresponds to the change in mass on the sensor surface. By immobilizing a target molecule to the sensor surface, and injecting 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. The dual channels give higher through-put and better referencing, simplifying crude samples analysis.

METHOD

Both assays used Attana's Amine coupling kit to immobilise antigen (60-100 Hz) on LNB-carboxyl surfaces.

Immobilisation was done in 10mM sodium acetate, pH 4.5 at a flow rate of 10 μ l/min and using 10mM HEPES 150mM NaCl, 0.005% Tween 20 (1xHBST) as running buffer. HBST buffer and a flow-rate of 25 μ l/min was used through-out the interaction part of the experiments.

In both experiments antigen (50 μ g/ml) was immobilised on the surface in channel A (sample surface), while the surface in channel B (reference surface) was only activated and de-activated. Sample was injected and the interaction dynamics monitored. The dissociation of the bound antibodies (hybridoma) or scaffold proteins (lysates) was sampled for at least five minutes. Buffer injections were used for referencing. Experiments were performed in order to control for avidity and non-specific binding.

Data were collected using the Attester software, and subsequently processed in the Evaluation software. The embedded off-rate screening tool in the Evaluation software was used to easily identify molecules with the lowest dissociation rate constants for the antigen.

RESULTS

Screening of *E. coli* lysates – 96 lysates containing single domain protein binding scaffold proteins were screened on an automated Attana 200 system (Attana A200). The lysates were injected in parallel over the sample (antigen immobilised) and the reference (activated/de-activated) surfaces.



After subtraction of the bulk response effects, as recorded on the reference surface (Fig. 1B), the specific interaction response curves were revealed (Fig. 1A-B) and could be used for

determination of rate constants. As seen in figure 1B (>100s) there is very low level of non-specific binding after the sample pulse has passed the reference sensor surface.

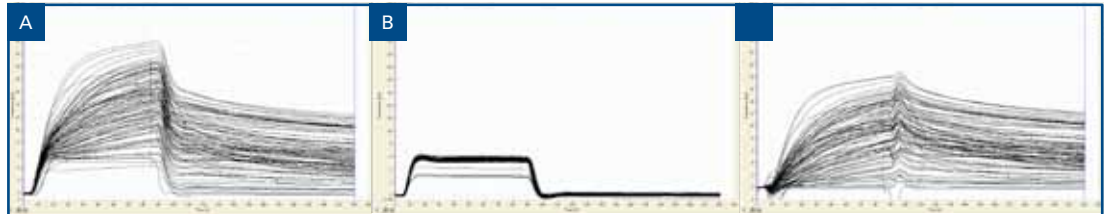


Figure 1:

Using the embedded off-rate screening tool of the Evaluation software, the injected molecules are readily ranked according to their respective dissociation rate constants from the antigen. Fig. 3A displays the off-rate screening list, highlighting the most stable interactions, as deduced from the dissociation rate constants. The list and graphics can be printed and exported in various formats.

200 system (Attana A200). The supernatants were injected in parallel over the sample (tagged antigen immobilised) and the reference (tag-only immobilised) surfaces.

Screening of hybridoma supernatants – Similarly to what has been described above, 25 serum containing Hybridoma supernatants with antibodies were screened on an automated Attana

After subtraction of the bulk response effects, as recorded on the reference surface (Fig. 2B), the specific interaction response curves were revealed (Fig. 2A-B) and could be used for determination of rate constants. As seen in fig. 2B there is, also here, very low level of non-specific binding after the sample pulse has passed the reference sensor surface.

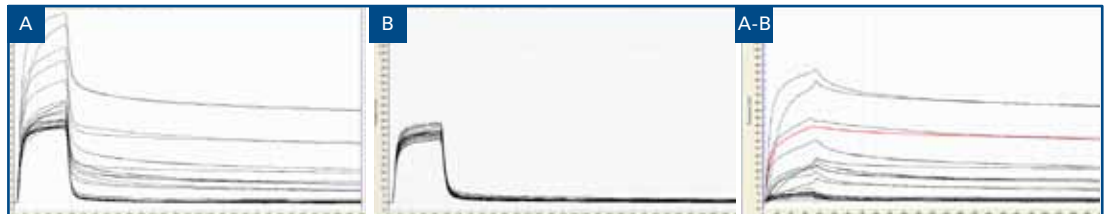


Figure 2: Screening and off-rate analysis of hybridoma supernatants. A) Supernatants interacting with antigen on the surface. B) Supernatants interacting with the reference surface A-B) Reference corrected data used for off-rate analysis.

The dissociation rate constants for the respective hybridoma supernatants were then directly determined from the resulting curves as described

above, and the samples ranked and displayed in the off-rate screening list (Fig. 3B).

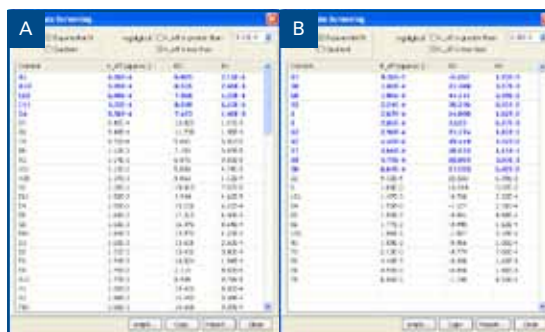


Figure 3: Off-Rate Screening window displaying a list of the analysed molecules ranked according to their dissociation rates. A) Bacterial lysates, Off-rates slower than $9.10 \cdot 10^{-4} s^{-1}$ are highlighted B) Hybridoma supernatants, Off-rates slower than $9.10 \cdot 10^{-4} s^{-1}$ are highlighted

Attana Materials Used	Item Code
Amine Coupling Kit	3501-3001
Attana® LNB Carboxyl Sensor Chip	3623-3033 (pack of 3)
	3623-3103 (pack of 10)
Attester™ software: 1.3.9.1	3440-3001
C-Fast software: 1.4.4	3450-3001
Evaluation software: 3.2.7.0	3460-3001

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