

Epitope Mapping – Sandwich Pair Selection



OBJECTIVE

Development of assay for studies of PSA monoclonal antibodies' binding characteristics.

CONCLUSIONS

- Attana® 100 can be used for cost efficient characterization of antibody-antigen interactions.
- The developed method allows affinity ranking of antibodies and early identification of reagents with good kinetic characteristics.
- The method is useful in early evaluation and selection of suitable antibody pairs for the design of sandwich immunoassays and for epitope mapping.
- The assay saves time compared to other immunochemical methods including cross-inhibition studies and permits the determination of dose-response curves and kinetic parameters.

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BACKGROUND

Immunological reagents with optimal recognition of antigen in solution are essential for immunoassay development. Recognition of independent epitopes and good kinetic properties are additionally important parts of a selection process for identification of optimal monoclonal antibodies for immunoassay development.

In the selection process for identification of optimal monoclonal antibodies for immunoassay development, analyses are typically performed using different immunochemical methods including cross-inhibition studies, determination of dose-response curves (for all possible antibody-antigen combinations) and analysis of binding constants. The use of the Attana 100 system enables label free, real-time analysis of antibody-antigen binding characteristics and provides kinetic data on the interaction. This application example provides a short description of strategies for selection and characterization of prostate specific antigen (PSA) monoclonal antibodies (mAbs) suitable for the design of immunoassays.

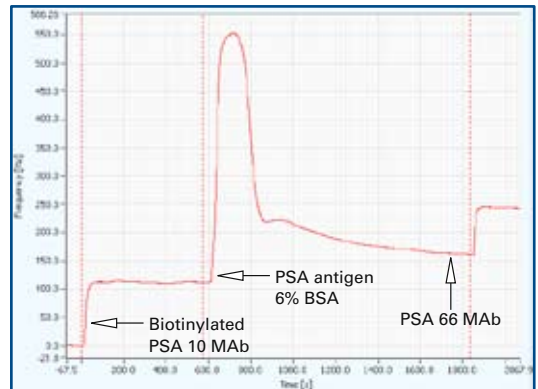


Figure 2: Binding events in the Attana 100 are shown as a negative frequency shift, whereas a positive frequency shift signifies desorption of molecules from the surface. The figure shows the immobilization of biotinylated PSA Antibody on an Attana Biotin Sensor Chip, followed by antigen binding and the subsequent binding of a different monoclonal PSA Antibody.

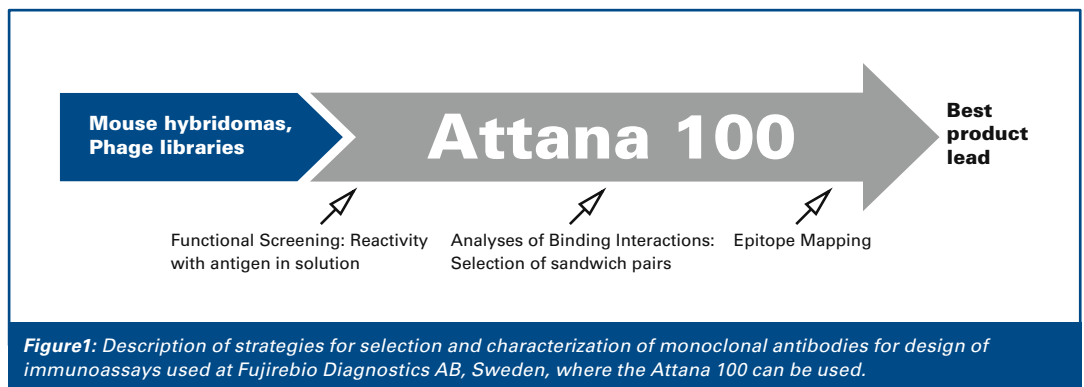


Figure1: Description of strategies for selection and characterization of monoclonal antibodies for design of immunoassays used at Fujirebio Diagnostics AB, Sweden, where the Attana 100 can be used.



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ATTANA 100 BIOSENSOR

The Attana 100 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 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 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

The binding of PSA mAb to PSA was studied to determine the relation between the antigenic domains recognized by different PSA mAb developed at Fujirebio. PSA was immobilized on the surface by biotinylated PSA10. Thereafter, antibodies in cell supernatant were added containing: PSA66 mAb, PSA30 mAb, PSA36 mAb, PSA27 mAb, PSA74 mAb. The different binding characteristics were then studied in real-time. Epitope mapping of different PSA mAb was also performed by immobilizing biotinylated PSA 10 mAb and injecting the PSA. Subsequent injections of PSA 36 mAb and PSA 30 mAb to the PSA were then monitored.

RESULTS

Screening of PSA mAbs from hybridoma supernatants: The results suggest that the combination of any of the tested PSA mAbs could be used for the development of PSA sandwich immunoassays. As the proteins are washed away by the buffer, the binding of the antibodies to the surface can be seen, i.e. in all samples the PSA mAb binds to the PSA antigen with varying affinity.

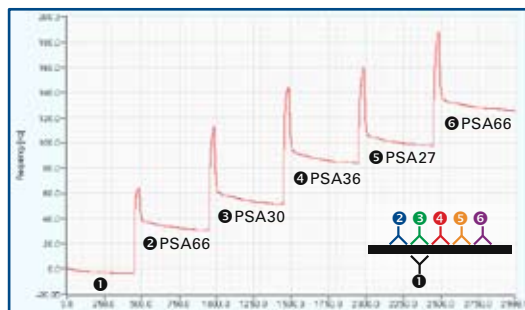


Figure 3: Sequential injections of different PSA mAb result in a decrease in frequency indicating that the mAb recognizes an independent antigenic domain in PSA (with courtesy of Fujirebio Diagnostics AB).

Epitope mapping of different PSA mAbs: As shown in **Fig. 4** the amount of bound PSA 30 mAb is decreased if other mAbs have already bound to the PSA. This may be interpreted as the previous mAbs either sterically interfere, or by induced antigen conformational changes, reduce available sites for the PSA 30. However, PSA 36 mAb binds to the antigen in the same way, irrespective if other mAbs are present.

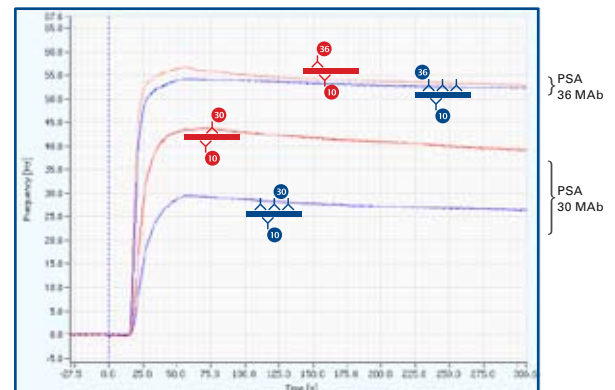


Figure 4: Sequences of mAb binding to PSA were studied for PSA 30 and PSA 36 to investigate interference between different mAbs in the binding to PSA. First, the PSA antigen was immobilized on a biotinylated PSA 10 mAb. Thereafter, the binding of the investigated mAb (PSA 30 and 36) was examined with and without potentially interfering mAbs.

Attana Materials Used	Item Code
Attana Biotin Sensor Chip	3613-3033 (pack of 3)
	3613-3103 (pack of 10)
Streptavidin	3502-3001
C-Fast: 3.1	3420-3001
Attester™: 3.1	3410-3001
Attester™ Evaluation: 3.1	3430-3001

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