Whitepaper

Rapid and Improved Immunoassay Development and Optimization Using the dotLab® mX System

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Background

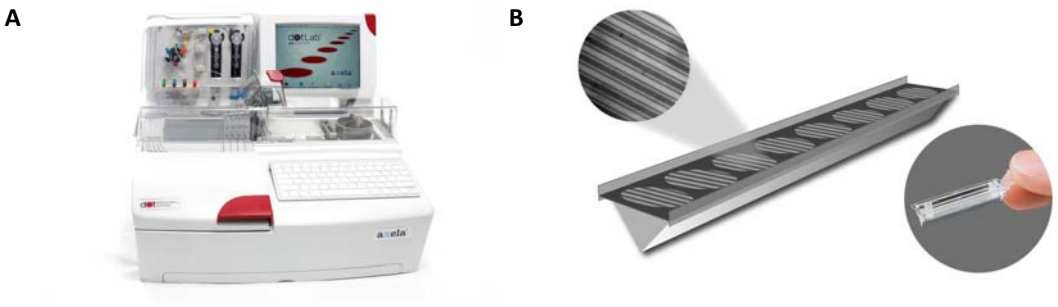
The development and optimization of immunoassays is a complex, multi-step process that is both time consuming and labor intensive to perform by traditional endpoint-based technologies. The dotLab® mX System can significantly simplify this process by providing real time interaction data on an easy to use, flexible and fully automated platform.

The dotLab® mX System

The dotLab® mX System utilizes diffraction-based optical sensing for the real time, label-free measurement of molecular interactions. The system uses inexpensive, disposable biosensors with coupling reagents (eg: avidin, amine reactive substrates or unique oligonucleotide-based addressing reagents to allow multiplexing) pre-patterned on the surface of 10 µL flow channels forming a diffraction grating. The dotLab® mX instrument illuminates the grating with a laser generating a diffraction image which is monitored by a photodiode detector. Diffractional efficiency increases as molecules bind to the surface resulting in an increase in image intensity. Conversely, molecular dissociation from the surface results in a decrease in image intensity. Therefore, the real time monitoring of molecular interactions through changes in diffractional efficiency provides information on the quantity and rate of binding and dissociation events. The dotLab® mX System simplifies and automates this analysis using a fully integrated, easy to use, bench top instrument.

Figure 1

(A) The dotLab® mX Instrument: a fully automated, bench-top instrument for real time molecular interaction analysis. (B) Schematic of a dotLab sensor with a contiguous array of capture surfaces (spots) with coupling reagent pre-patterned on the surface forming diffraction gratings.



Here, we demonstrate the use of the dotLab® mX System to facilitate various steps in the immunoassay development and optimization process.

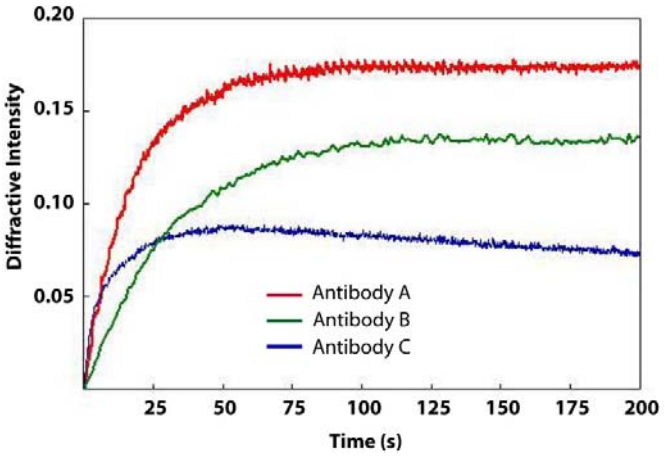
1) Antibody Ranking

The first step in immunoassay development is the acquisition and characterization of antibodies against the target antigen. Whether antibodies are purchased commercially or generated in house, their antigen binding properties need to be evaluated to make the appropriate

selection. In Figure 2, the antigen binding properties of three candidate antibodies were compared using the dotLab® mX System. The relative association rate, levels of binding at equilibrium and time to reach equilibrium of the antibodies can rapidly be deduced and compared . Endpoint methods such as ELISA only provide information on the levels of binding after a fixed incubation period. Thus, the real time analysis provides additional information on antibody binding characteristics that can be used for antibody selection.

Figure 2

Antibody Ranking. A biotinylated antigen was immobilized on avidin-coated sensors and then incubated with equal concentrations of three candidate antibodies. The binding characteristics of each of the antibodies to the immobilized antigen can be rapidly deduced and compared.

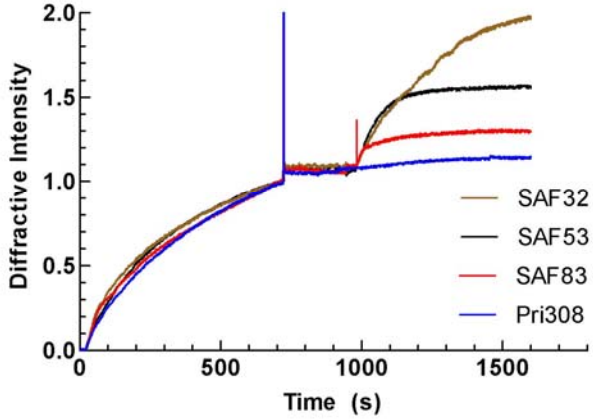


2) Antibody Pairing

The development of sandwich format immunoassays requires the selection of two antibodies that not only demonstrate good binding to the target antigen independently, but also show good performance when used together. Figure 3 shows the binding of four secondary antibodies to an antigen bound to an immobilized capture antibody on the dotLab® mX System. The real time analysis of secondary antibody binding

Figure 3

Antibody Pairing. A biotinylated capture antibody against prion protein, PrP (3F4) was pre-mixed with PrP and applied to avidin-coated sensors (A). The binding characteristics of four secondary antibodies, SAF53, SAF32, DAF83 and Pri308 to the 3F4 bound PrP were then evaluated to determine the optimal antibody pair for use in a PrP sandwich immunoassay (B).



yielded their relative binding rates, time to reach equilibrium and binding levels at equilibrium. Based on these parameters, the appropriate secondary antibody for use with the capture antibody was selected. Interestingly, the aberrant shape of the brown binding curve was later shown to be due to the recognition of that antibody to an octo-repeat region of the antigen resulting in multiple binding events per molecule.

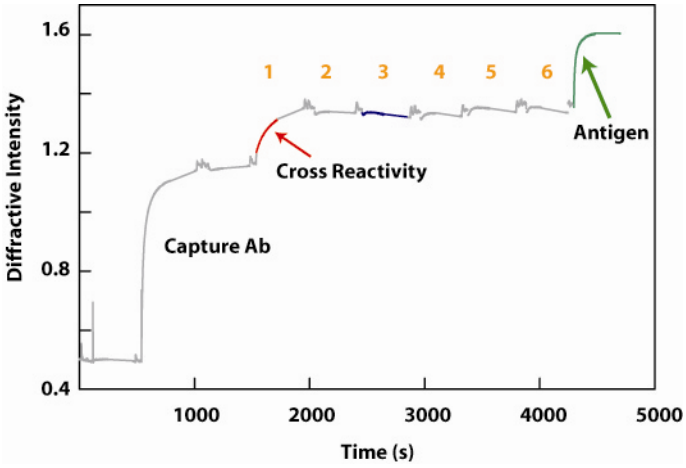
3) Cross Reactivity Analysis

Cross reactivity between reagents used in an immunoassay can lead to undesired noise and background signal. The detection and monitoring of conditions to minimize reagent cross reac-

tivity is a time consuming, labor intensive process and can unnecessarily consume large quantities of samples. In Figure 4, the cross reactivity of several reagents to a capture antibody was investigated in a single assay using the dotLab® mX System.

Figure 4

Cross Reactivity Analysis. The possible cross reactivity of six reagents to a capture antibody was evaluated. Biotinylated capture antibody was immobilized on an avidin sensor, followed by the sequential incubation of each of the six reagents with brief washes between incubations. The results showed cross reactivity between reagent 1 and the capture antibody while reagents 2-5 exhibited no cross reactivity. Following reagent 6, the sensor was incubated with antigen as a positive control.



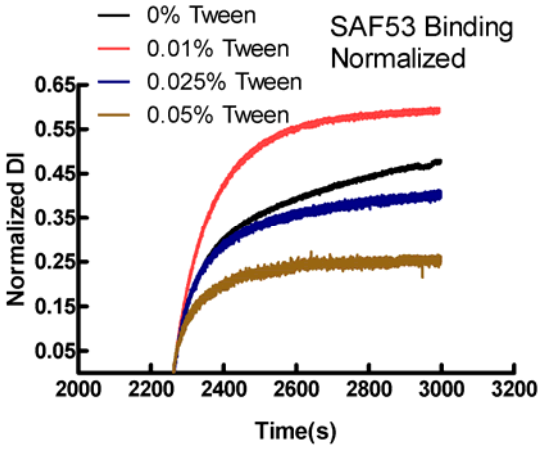
Using this approach, the potential cross reactivity of many reagents can be tested in a single assay, significantly simplifying and speeding up the process. Moreover, once identified the effectiveness of the measures to block the cross reactivity can be monitored using the dotLab® mX System.

4) Buffer Optimization

The determination of assay conditions that are optimal for the desired interactions while minimizing background signal and cross reactivity represents a significant challenge during immunoassay development. The use of detergents may assist in minimizing these undesired interactions. However, antibody-antigen interactions can be disrupted by high concentrations of detergent. Thus, the determination of optimal detergent concentration is important for optimal immunoassay performance. Figure 5 shows the binding of an antibody to an immobilized antigen performed in a buffer supplemented with various concentrations of detergent. Based on the binding characteristics of the antibody, the optimal detergent concentration can be quickly deduced. In a similar manner, other buffer parameters such as pH and salt concentration can be rapidly optimized.

Figure 5

Buffer Optimization. The effects of various concentrations of Tween 20 on antibody-antigen binding were evaluated. Recombinant PrP was immobilized on avidin-coated sensors and then incubated with SAF53 antibody in PBS containing various concentration of Tween 20. The results showed that the highest levels of SAF53 antibody binding was observed with 0.01% Tween 20.



Summary and Conclusions

- The label-free real time detection of protein interactions using the dotLab® mX System enables the rapid and simple screening of immunoassay reagents and optimization of assay conditions.
- This reduces immunoassay development time, sample consumption and improves overall assay performance.
- Real time interaction data provides information that is not available by endpoint-based methods such as ELISA, enabling the user to make better informed decisions on assay parameters.
- Flexible assay formats and broad dynamic range of detection make the dotLab® mX an excellent platform to run routine immunoassay.
- The use of Axela's panelPlus™ addressing reagents[1] enables the development and running of multiplexed immunoassays on the dotLab® mX System.

References

1. Lin et al. (2009) Intra-feature and inter-feature multiplexing using diffractive optics technology: more information from less sample. Presented at the 41st annual Oak Ridge conference.

About Axela, Inc.

Axela's platforms provide powerful new approaches to multiplexed protein and nucleic acid analysis designed to greatly simplify biomarker testing in clinical research and diagnostics. Axela's commercial research products significantly improve the amount and quality of information derived from traditional assays. This approach shortens time to result and provides access to unique categories of markers that form a pipeline of future diagnostic offerings.

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