Characterization of Therapeutic Antibodies using Digital SPR

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Introduction

Alto is the world's first SPR instrument to integrate digital microfluidics (DMF) with nanotechnology-based biosensors. With simultaneous detection across 16-channels, Alto provides high-throughput analysis of up to 48 unique targets, while further streamlining user workflows with automated sample dilutions, disposable fluidics, and sample requirements reduced by up to 200X.

Here, we demonstrate how Alto is an ideal platform for therapeutics development through crude library screening, binding kinetics characterization, and epitope binning of influenza antibodies.

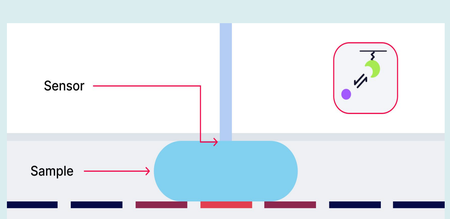
Alto™ Digital SPR: Label-free binding analysis in a droplet

Nicoya's Alto system uses digital microfluidics (DMF) to deliver automatically diluted sample droplets to SPR sensors for effortless real-time characterization of biomolecular interaction analysis including quantitation, screening, epitope binning and binding kinetics.

- 16 independently addressable channels for high throughput.
- Complete kinetics analysis using only 2 µL of crude or pure sample.
- No manual dilutions, tagging, degassing, cleaning, or strenuous assay set up.
- Powerful epitope binning, data visualization and analysis software.

What is DMF? (Digital Microfluidics)

DMF is a liquid-handling technology capable of accurately controlling and manipulating discrete nanoliter-sized droplets across an array of electrodes. The fluidics are contained within a disposable cartridge, allowing Alto to overcome the major limitations associated with increasingly complex fluidic systems present in traditional label-free instruments.



Library Screening

Real-time, label-free binding analysis is routinely used to screen hybridoma clones for high affinity antibodies prior to further expansion and subsequent characterization. Alto allows for rapid, automated, SPR-quality screening and characterization of biologic lead libraries with simple, pre-configured capture screening and binding kinetics characterization assays to select lead molecules.

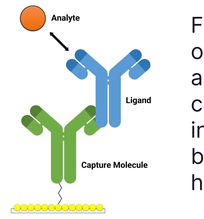


Figure 1 shows an example of a screening assay using an anti-IgG surface to capture anti-HA antibodies in serum followed by binding to the hemagglutinin (HA) antigen.

A

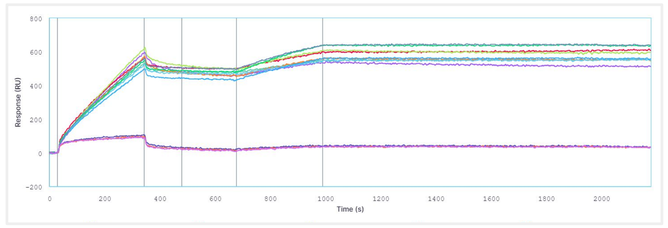


Figure 1: A) Series of sensorgrams showing the capture (or absence of capture) of the ligand followed by washing with buffer and then binding (or absence of binding) of the HA antigen

B

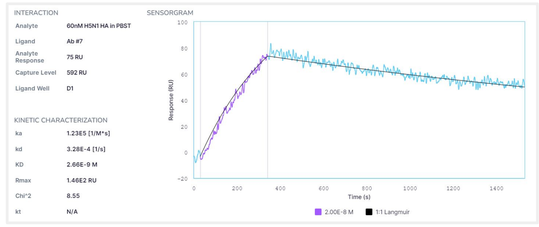


Figure 1: B) Sensorgram for a single antigen interaction with capture level, analyte response and kinetic parameters

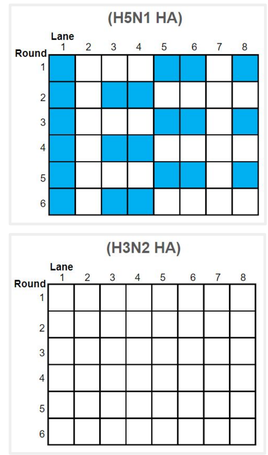


Figure 1: C) Heatmap showing specificity of antibodies against the H5N1 HA (top) and the H3N2 HA antigens (bottom). Blue indicates wells containing captured anti-HA antibody and binding to the HA protein and white indicates no binding was detected.

Kinetics Characterization

Alto offers powerful time, labor and reagent savings for running traditional multi-cycle (MCK) or single-cycle kinetics (SCK) on 48 unique interactions per cartridge. For each analyte, Alto measures five (5) unique concentrations by automatically performing 3X serial dilutions.

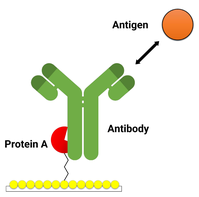


Figure 2 shows an example of single-cycle (SCK) and multi-cycle (MCK) binding kinetics data for the HA antigen binding a panel of anti-HA mAbs captured across 8 sensors in parallel on Protein A biosensors.

A

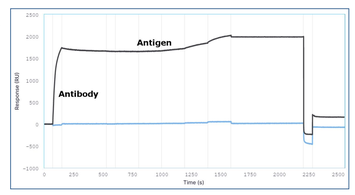
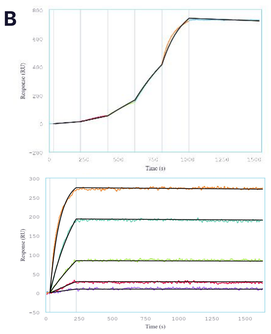


Figure 2: A) Raw SCK data showing the 5 concentrations of HA antigen passing over the captured anti-HA mAb surface (black curve) and the reference sensor (blue curve). Binding kinetic rate constants and affinity constants for all 48 unique mAbs were measured in a single unattended run using just 20 ng of each mAb and < 1 µg of antigen.

B



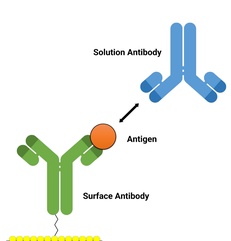
C

Cat #	K_D (M)	Cat #	K_D (M)
RM08	5.53E-12	**RM07**	6.02E-11
MM14	7.18E-12	**MM10**	1.10E-10
MM01	1.10E-11	**RM02**	1.37E-10
MM11	1.13E-11	**MM08**	1.73E-09
MM03	1.22E-11	**RM06**	3.11E-09
RM09	1.33E-11	**MM05**	3.15E-09
MM04	1.35E-11	**RM01**	6.54E-09
MM06	2.16E-11		

Figure 2: B) Examples of reference corrected SCK and MCK curves of HA antigen binding to the captured anti-HA mAb. Black curves shows the 1:1 fit overlay. **C)** Summarized table of binding profiles for 15 unique mAbs binding to HA antigen.

Epitope Characterization

Alto allows users to design a 16×16 assay to characterize the simultaneous binding of mAbs to an antigen, tested in a pairwise manner. In the classical sandwich assay format, antigen is captured by up to 16 unique surface coupled antibodies, which is followed by the pairwise binding of solution antibodies. Alto uses only 100 ng of each antibody for the entire experiment.



An antibody that blocks another antibody from binding to the antigen will be deemed to have the same target epitope and will be 'binned' together. If both antibodies bind the antigen at the same time it can be inferred that they have non-overlapping epitopes.

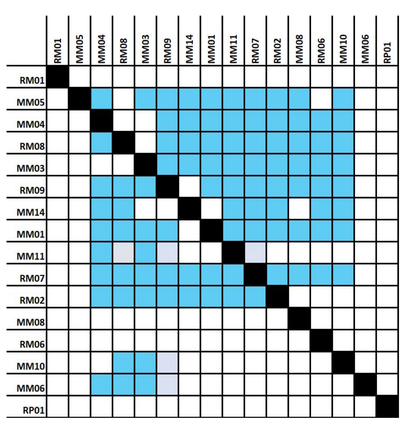


Figure 3: Heat map of 16×16 epitope binning of anti-HA antibodies against the HA antigen, with blue indicating 'bind'.

Conclusion

Alto facilitates crude sample screening, accurate kinetics analysis, and 16×16 epitope binning for accelerated development of antibody therapeutics. By leveraging DMF technology, Alto streamlines SPR analysis by automating sample dilutions, eliminating fluidic maintenance, and reducing sample requirements by up to 200X.

Learn how Alto can take your discoveries to the next level. Visit our website or contact us:

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