Label-free screening and selection of multiple antibody-antigen pairs for assay development using the Octet System

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ABSTRACT

One of the challenges in developing immunoassays is to identify suitable antibody pairs that recognize the same target to non-overlapping, non-interfering epitopes. Selection of the most robust antibody pair requires evaluation for both specificity and affinity, which can be time consuming and difficult to determine with current methods. The Octet System provides a rapid analytical method to screen antibody pairs under label-free, real-time conditions. Processing capabilities of 8 assays in parallel, combined with real-time information on the antibody-analyte binding provides the researcher the ability to quickly determine affinity and specificity for 8 different putative antibody pairs for a single target.

Identifying suitable antibodies to pair in immunoassays is often challenging, particularly for sandwich ELISAs whereby two antibodies bind to the same analyte. In this type of assay, one antibody is used to capture the analyte and a second antibody is used for detection. Selection of antibody pairs must be evaluated for the following characteristics:

- Antibodies must bind to non-overlapping, non-interfering epitopes
- Antibody-analyte interactions must be of high enough affinity to withstand wash protocols
- Capture and detection antibodies must exhibit a high degree of specificity

Octet System for Antibody Pair Selection in Assay Development

The Octet System provides a rapid analytical method to screen antibody pairs. It gives information on the antibody-analyte binding interaction in an intuitive real-time format. Combined with parallel processing of 8 samples, the researcher can quickly identify antibody pairs for further development. In addition, as a label-free detection system, screening can be performed on a panel of antibodies without labor intensive labeling; thus saving time and resources.
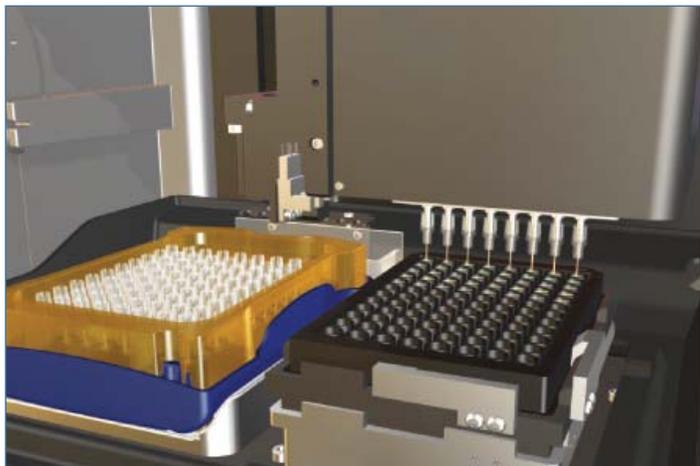

FIGURE 1: ForteBio's Octet System.

Measuring Protein Interactions on the Octet System Using BioLayer Interferometry (BLI) Technology

Octet provides real-time monitoring for protein: protein interactions and binding events using BioLayer Interferometry (BLI) technology. Any change in the number of molecules bound to the biosensor tip changes the optical layer thickness. Changes in optical thickness cause a shift in the interference pattern (Δλ)that can be measured in real time (Figure 2).
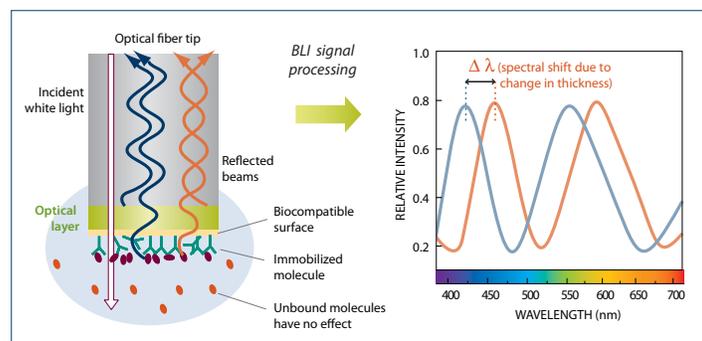

FIGURE 2: Principle of Octet BioLayer Interferometry (BLI) technology.

Adding molecules (binding) increases the thickness of the biological layer; shifting the wavelength peaks to the right. Removing molecules (dissociation) reduces the thickness of the layer and shifts the wavelength peaks to the left. The wavelength shift (Δλ) is a direct measure of the change in thickness (nm) of the biological layer.

Assessing a Sandwich Assay Using BioLayer Interferometry

Protein expression campaigns produce hundreds of clones. Screening techniques can be labor-intensive, and clonal selection can become a bottleneck in the development process. In less than 30 minutes, the Octet System can quickly identify which clones are expressing at high to moderate levels.
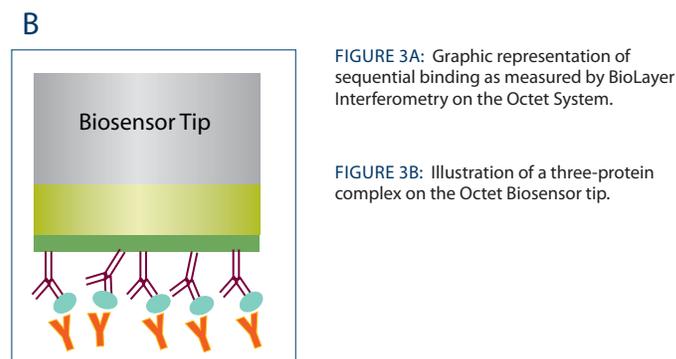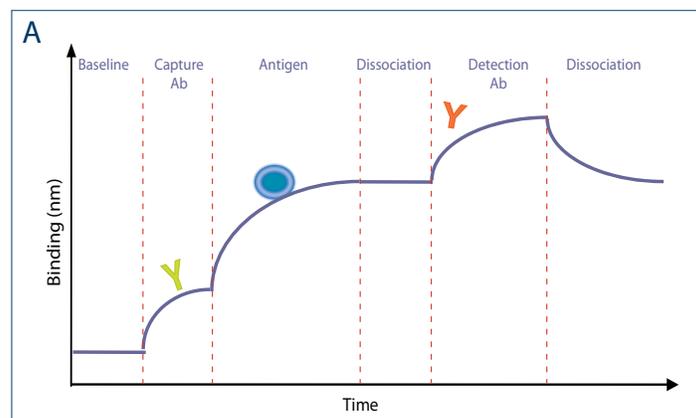

FIGURE 3A: Graphic representation of sequential binding as measured by BioLayer Interferometry on the Octet System.

FIGURE 3B: Illustration of a three-protein complex on the Octet Biosensor tip.

Addition of a dissociation step allows for estimation of the overall affinity of the three-protein complex. By varying the buffer conditions during the dissociation steps, wash conditions can be screened to maximize positive signal while minimizing non-specific binding. Screening for viable antibody pairs in this fashion enables the user to evaluate the specificity and strength of each interaction. This is in contrast to standard approaches in which decisions on which pair to develop are made based on a single end point measurement.

Rapid Screening of Antibody Pairs Using BioLayer Interferometry

Parallel processing of 8 biosensors on the Octet System provides simultaneous screening of 8 different putative antibody pairs for a single analyte. Two antibodies were assayed in parallel against a single analyte with subsequent binding to 4 different detection antibodies.
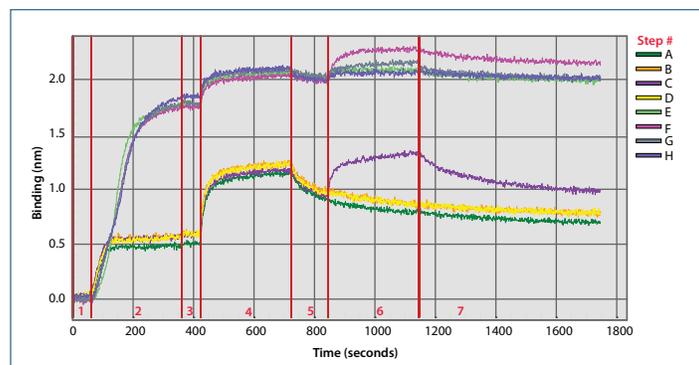

FIGURE 4: Real-time binding chart of sequential binding of 2 antibodies to an analyte and subsequent binding to 4 different detection antibodies.

Figure 4 illustrates the real-time binding chart of the antibody pair screen. Two pairs can be quickly identified for further assay development. Ab1 capture antibody with detection antibody Ab5 exhibits good binding to the analyte. The interaction between Ab1/Ab5 also exhibits some dissociation, indicating a weaker affinity. Ab2 capture antibody with Ab4 detection antibody exhibits both good binding and good affinity.

STEP						
1	2	3	4	5	6	7
PBS	Capture antibodies Ab1 A, B, C, D Ab 2 E, F, G, H	PBS	Analyte	PBS	Detection antibodies Ab3 A, E Ab4 B, F Ab5 C, G Ab6 D, H	PBS

TABLE 1: Sample plate assay setup for antibody pair indentification on the Octet System using Streptavidin SBC Biosensors.

Step	Description
1	Baseline of Streptavidin Biosensors.
2	Biotinylated capture antibodies bind to Streptavidin. Biosensors A–D represent Ab1 and Biosensors E–F represent Ab2.
3	Baseline of immobilized capture antibodies.
4	The analyte of interest binds to the capture antibodies.
5	Stability of the analyte-capture antibody interaction is assessed by examining the dissociation curve in buffer. Ab1 exhibits a faster off-rate than Ab2.
6	A set of 4 detection antibodies are tested against both Ab1 and Ab2.
7	Stability of the sandwich protein complex is monitored in buffer.

TABLE 2: Description and analysis of an antibody pair screen of 2 capture antibodies against 4 detection antibodies to a single analyte.

Antibody Isotyping Application on the Octet System

One application for sandwich type assay formats is the isotyping of monoclonal antibodies in the presence of background proteins. The Octet System provides a simple method for rapid identification of both the heavy and light chain subclasses.

in Figures 5 and 6, a biotinylated anti-mouse Fc antibody was immobilized onto streptavidin biosensors. The biosensors were then used to bind the mIgG (10µg/mL) to be isotyped out of a sample with high protein background (>1 mg/mL). After a brief wash in buffer the biosensors were exposed to a series of isotype specific antibodies as denoted in the legend. By taking advantage of the Octet's ability to parallel process up to 8 samples simultaneously, a panel of isotype specific antibodies were used to detect the mIgG's isotype in ~15 minutes.
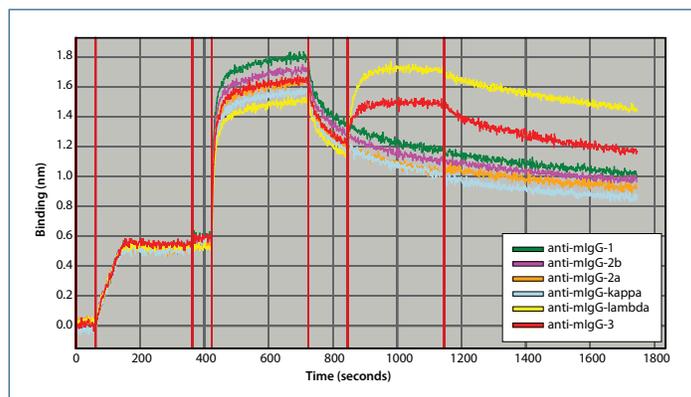

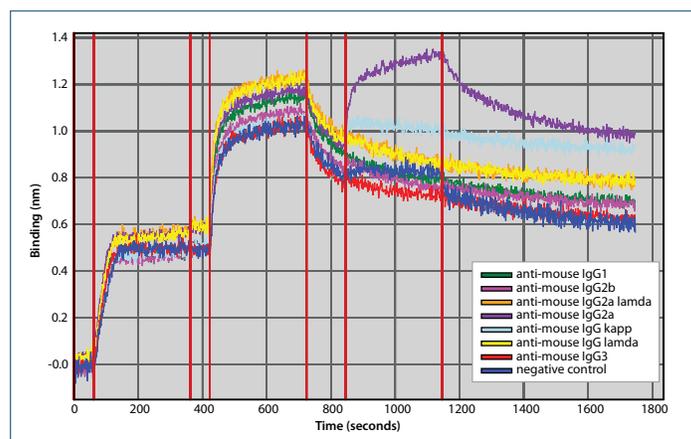FIGURE 5: Isotyping of a mIgG3 lamda antibody on the Octet System.

FIGURE 6: Isotyping of a mIgG3 kappa antibody on the Octet System.

Figure 5 clearly shows that the isotype antibodies which bound correspond to the anti-IgG3 and the anti-lamda antibodies. This correctly identifies the mIgG from the sample as a mouse IgG3 lamda. Figure 6 shows the real time binding chart from an assay in which a panel of 7 isotype specific antibodies were used simultaneously. From the pattern of the binding, the mIgG sample was correctly identified as mIgG2a kappa.

Summary

With an easy to use, flexible platform design, the Octet System streamlines antibody pair development by resolving crucial questions to produce more robust antibody pairs that are highly specific with high affinity for both the capture and detection antibody. In particular, the Octet excels at:

- Screening for capture and detection antibodies which bind to the analyte.

- Screening for specificity by varying the only the analyte.

- Screening putative antibody pairs for highest affinity and stability of the antibody-analyte-antibody complex.

- Identify antibody epitopes recognition based on an epitope panel.

- Screening antibody pairs for effective wash protocols that may affect affinity.