Label-free screening strategies using the Octet System from ForteBio

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ABSTRACT

The flexibility and throughput of ForteBio's Octet system lends itself readily to a variety of screening applications which will be discussed in detail.

Clonal screening and selection based on protein expression levels can be an important step in antibody development. Using Protein-A Biosensors, the Octet System can screen 96 samples in 30 minutes to provide accurate quantitation from crude lysates or media samples.

Dissociation-rate screening to rank protein affinities is critical to the development of therapeutic antibodies. Using five-minute off-rates as a selection criterion, 96 samples can be screened against a biotinylated target in little over an hour.

Affinity screening on Octet is convenient and rapid and can provide accurate determinations of protein kinetics with a minimum of development time. Using Octet to screen lysates against a series of amine-coupled targets in a multiplexing strategy further accelerates the selection of potential drug candidates..

Octet System for Protein Therapeutic Development

The Octet System improves the workflow for protein kinetics and kinetic screening by combining ready-to-use biosensors and sensitive optical detection with user-friendly, intuitive software. Data is presented in real-time and proteins can be analyzed without using labels.

The Octet System operates with an 8-channel biosensor manifold that can process up to 8 samples in parallel with a maximum throughput of 96 sensors for kinetic screening. The flexibility of assay set-up allows for full online kinetic characterization or off-line batch immobilization for screening online.

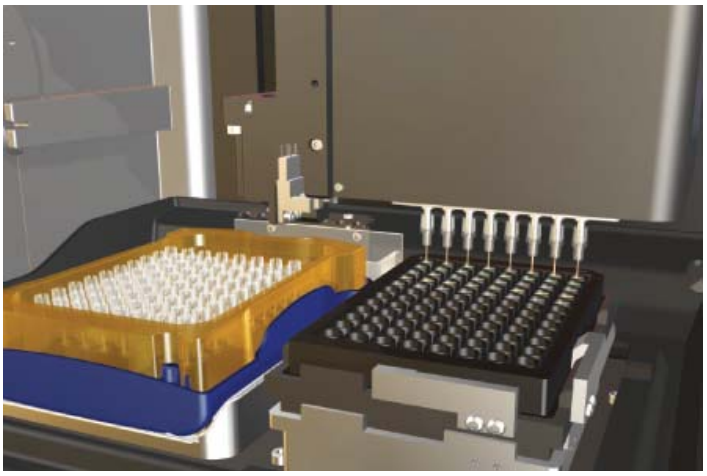

FIGURE 1. ForteBio's Octet System.

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 that can be measured ($\Delta\lambda$) in real time.

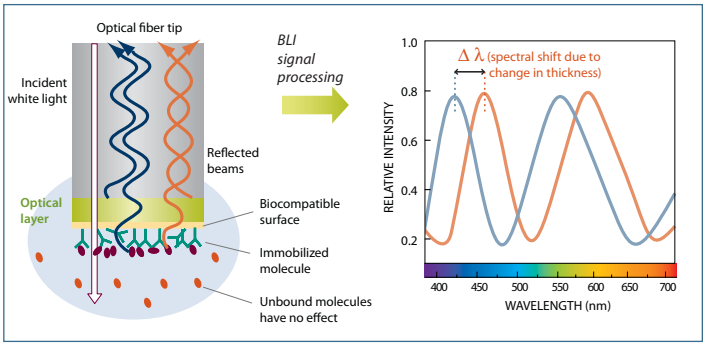

FIGURE 2. Principle of Octet BioLayer Interferometry (BLI) technology.

Adding molecules (binding) increases the thickness to 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 ($\Delta\lambda$) is a direct measure of the change in thickness (nm) of the biological layer. A change in optical mass thickness of 1 nm is equal to 1 nm shift in the interferometry wave pattern.

Rapid Screening of Clones

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.

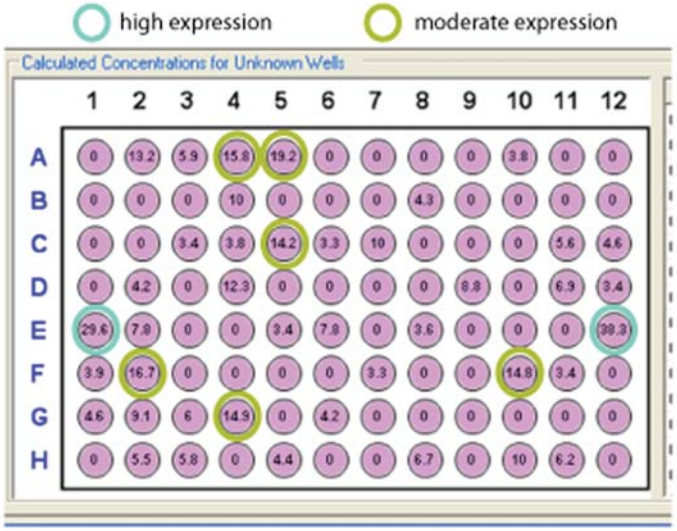

FIGURE 3: Octet System software quantitation results presented in a plate format.

By using either the anti-human IgG or Protein A sensors in the Quantitation mode, real-time binding charts and the subsequent software analysis provide a solution to quickly screen antibody clones for expression.

Figure 3 is an illustration of the screen display of a results plate map from the Octet System run in the Quantitation mode. High expression (teal) and moderate (green) is highlighted, but they can be quickly identified by sorting the results table in descending order.

The experiment consisted of screening of 96 clones for expression in a single run on the Octet System. Higher expression is easily identified by a large nm shift in the real-time binding chart (Figure 4).

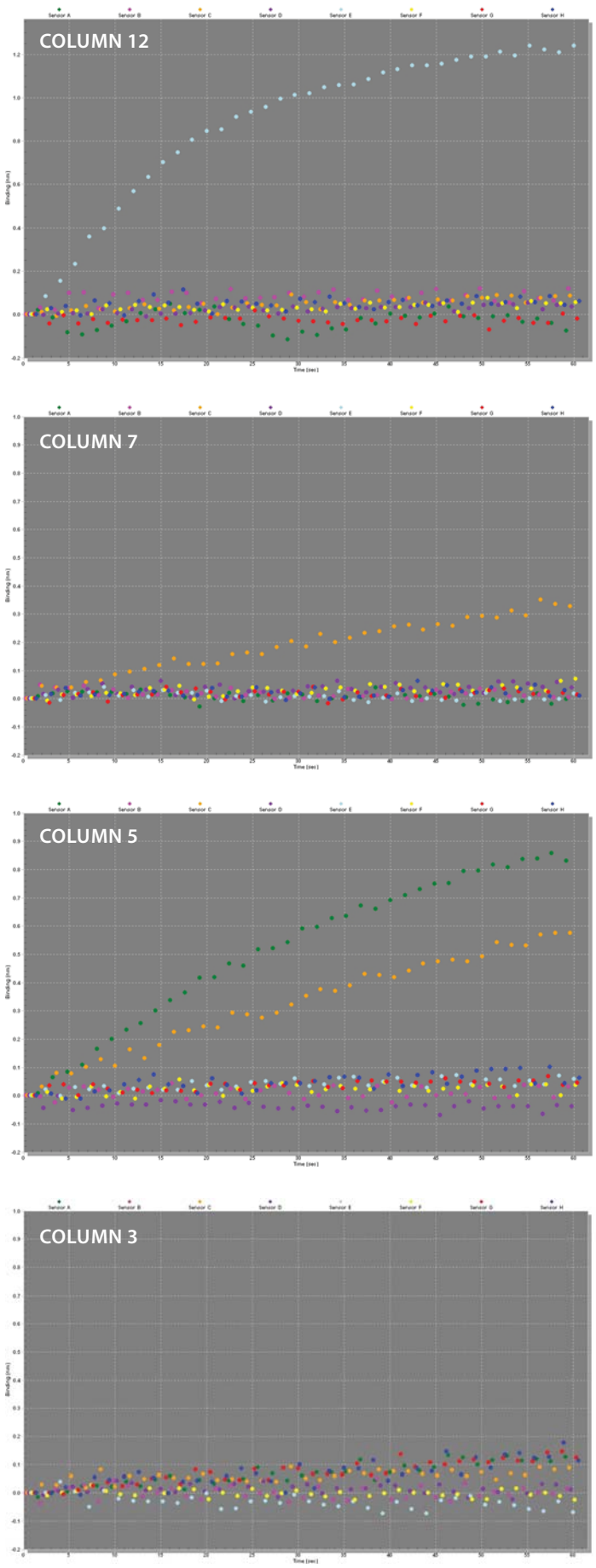

FIGURE 4: Real-time binding charts in the Quantitation mode facilitates identification of clones with higher expression

Affinity Screening for Receptor:Ligand Interactions

The Amine Reactive Biosensors provide a rapid solution to screen for the affinity of receptor:ligand interactions. In the screening assay shown below, a purified receptor was immobilized onto the amine reactive sensor and assayed against twelve ligands in duplicate.

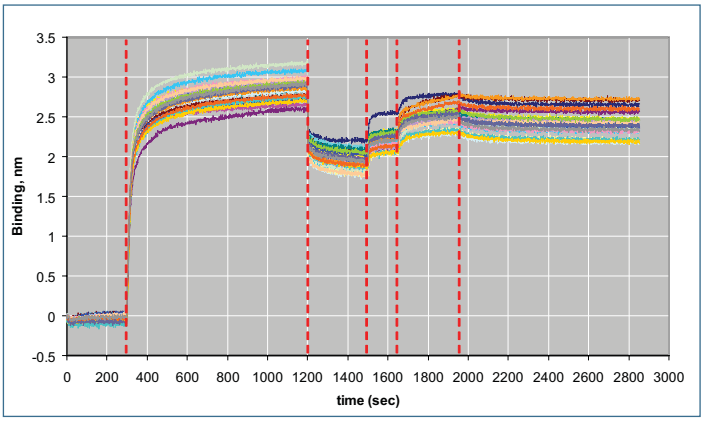

FIGURE 5: Real-time binding chart of immobilization of the receptor and subsequent binding of ligands.

Immobilization of the receptor exhibited good reproducibility with a CV of 4.8%, N=24.

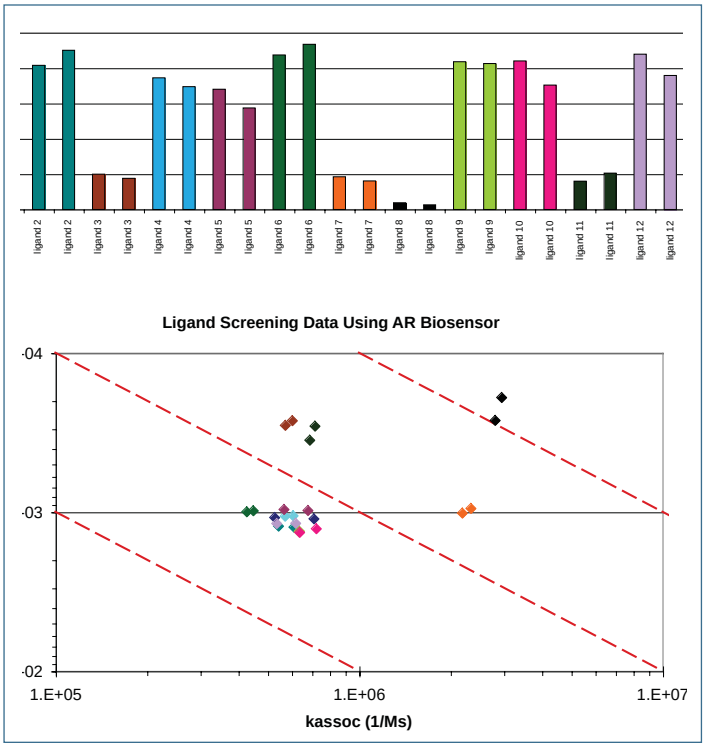

FIGURE 6: Kinetic characterization of the receptor:ligand interaction using the Amine Reactive Biosensors on the Octet System. The affinity (KD) bar chart and kdissoc/kassoc scatter plot are presented.

The receptor:ligand screen illustrated good reproducibility between replicate kinetic values for all twelve ligands. Figure 6 illustrates how tight binders can be quickly identified in a screen.

Kinetic Screening and Selection

The Octet System provides a solution to rapidly screen and identify binding interactions between antigen:antibody pairs. In the same experiment, the off-rates can be determined and subsequently ranked to select for promising clones to advance in the development process.

Using Streptavidin Biosensors on the Octet System, a biotinylated antigen was immobilized onto the sensor surface offline. Twenty-two clones were screened against the antigen for binding and subsequent off-rate analysis.

The clones were allowed to bind for 500 seconds and a 300 second off-rate was subsequently assayed in a single run. The Octet System samples 8 sensors in parallel for all kinetic assay steps. Figure 7 is the actual real-time kinetic binding charts for 3 sets of eight sensors sampled, N=22. Figure 8 exhibits the Octet System Software calculated graphs of the off-rates (kd); whereby, the rank order of the clones were rapidly identified.

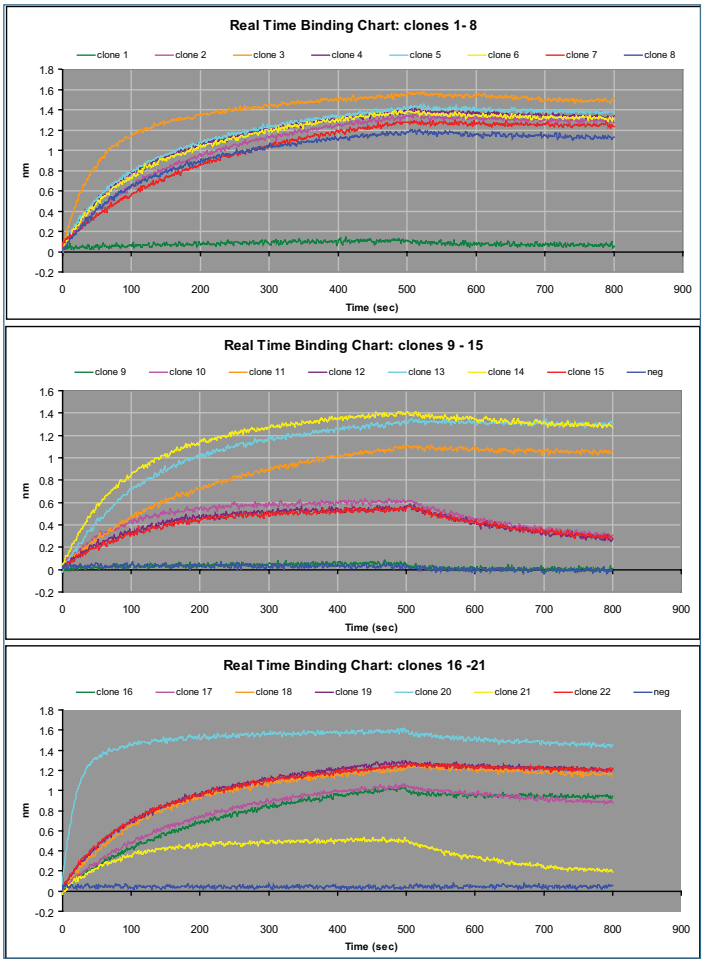

FIGURE 7: Real-time binding charts of each series of 8 sensors that comprise the screen of 22 clones to the biotinylated antigen.

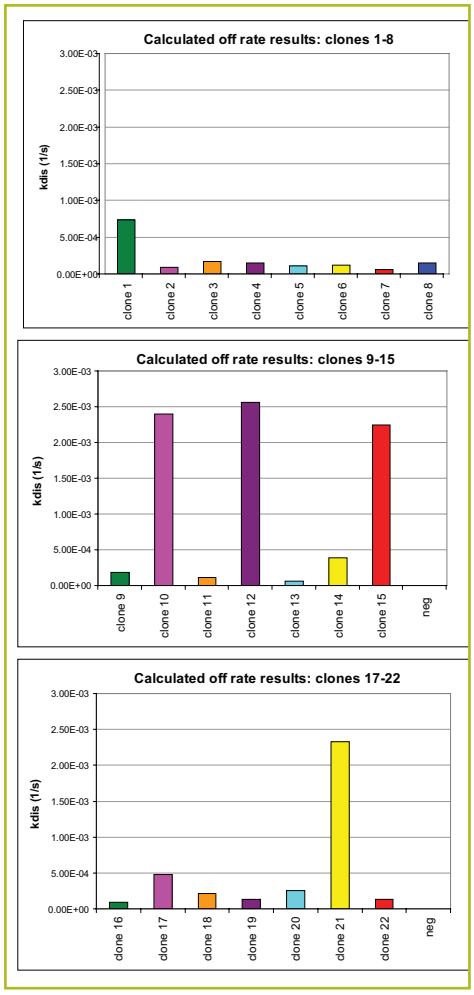

FIGURE 8: Matched off-rate charts of the screen of 22 clones to the biotinylated antigen.

Summary

ForteBio's Octet system for label-free real-time analysis of molecular interactions can be applied to a variety of screening applications. With either quantitation or kinetic modes, screening strategies faciliate rapid therapeutic drug development.

- Screening for expression of clones using the Anti-human IgG biosensor is quickly determined in the quantitation mode.
- Screening for receptor:ligand binding and subsequent affinity ranking using Amine Reactive Biosensors in the kinetics mode.
- Rapid screening of antibody clones for off-rate using Streptavidin biosensors in the kinetics mode.

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