ForteBio's Octet - A Novel System for Measuring Protein Interactions in Antibody Development and Production

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ABSTRACT

The efficient development and production of antibodies requires analytical methods that are robust, accurate and real-time. The methods should also be simple to run, require minimal training and fit with the workflow of antibody production.

The Octet system uses biosensors in a 96-well plate format to report quantitation and kinetic analysis. The system can analyze crude media and lysates streamlining the workflow of monitoring antibody production from bioreactors. The rapid reporting can provide information on breakthrough when loading purification columns with culture supernatants.

Octet can provide rapid kinetic analysis or affinity screening for antibody characterization at any stage in the production process. The dissociation rates of 96 antibody candidates can be rank-ordered in less than one hour with full analysis and reporting.

Octet System for Antibody Development

Current analytical methods for antibody bioprocessing include HPLC and ELISA, which are time consuming and technician-dedicated methods that impact workflow, hands-on time and cost. As a label-free, real-time detection system, the Octet accommodates applications at all stages of antibody and protein development and extends to capabilities that current methods do not support.

Figure 1. Octet System

By combining ready-to-use biosensors and sensitive optical detection with user-friendly, intuitive software, the Octet System improves the workflow for protein quantitation, protein kinetics and kinetic screening. Protein binding charts are presented in real-time. For protein quantitation, the 8-channel biosensor manifold can process 96 samples in 20 minutes. Kinetic results are wholly dependent on dissociation rates, but with 5-minute off rates for affinity ranking, 96 samples can be processed in 1 hour.

Measuring Protein Interactions on the Octet System using BioLayer Interfereometry (BLI)

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 (Δλ) in real time.

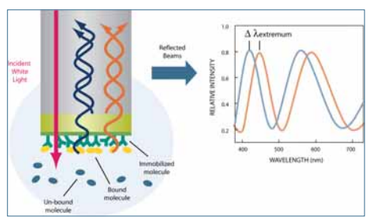

Figure 2. Octet BioLayer Interferometry Technolgoy

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 (Δλ) is a direct measure of the change in thickness (nm) of the biological layer.

Octet System: Quantitation and Workflow

Accurate antibody quantitation is critical to the selection of cell lines for development and optimization of yield and purification. Compared to using ELISA or HPLC, the Octet System measures direct antibody binding in a precise and rapid assay. The comparison assumes a typical sample batch of 50 samples *(Figure 3)*.

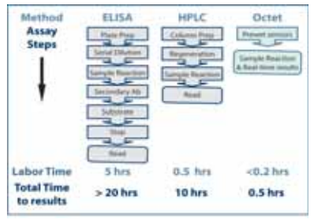

Figure 3. Method comparison for antibody binding

Assay Principle and Data

Antibody concentration is determined by measuring the rate of binding to a capture molecule under a set of standard conditions.

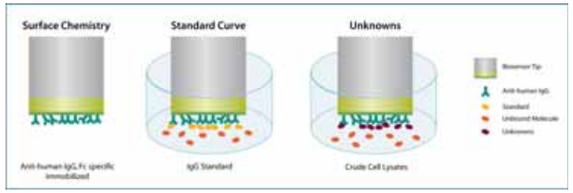

Figure 4. Surface chemisty and automated assay workflow for quantitation on the Octet

Binding curves for each standard are generated in real-time *(Figure 5)*. Upon completion, a standard curve *(Figure 6)* is automatically derived from the different binding rates and used to calculate the concentration of unknown samples according to user-defined curve fit parameters.

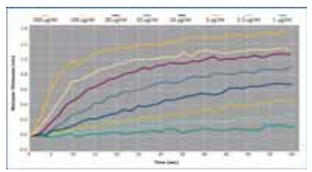

Figure 5. Octet real-time binding curves

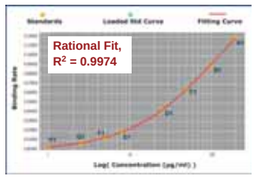

Figure 6. Octet standard curve

Correlation to HPLC

Antibody quantitation from the Octet correlates well with HPLC results.

Antibody samples ranging from 50 mg/mL to 15 mg/mL as determined by HPLC were assayed on the Octet .

Octet correlation to HPLC is greater than 0.95. *(Figure7)*

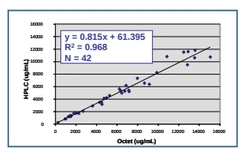

Figure 7. Octet correlation to HPLC

Crude Lysates

One of the limitations of HPLC is the inability to assay crude samples such as uncleared lysates. IgG expressed in E. coli was monitored by HPLC and Octet. Octet quantitation of both centrifuged and crude lysates correlated well with HPLC data. (Table 1)

HPLC Centrifuged	Octet Centrifuged	Octet Uncleared
0 µg/ml	0 µg/ml	0 µg/ml
248 µg/ml	280 µg/ml	281 µg/ml
31 µg/ml	42 µg/ml	47 µg/ml
333 µg/ml	323 µg/ml	327 µg/ml
100 µg/ml	132 µg/ml	130 µg/ml

Table 1. Octet & HPLC quantitation of lysates

Octet System: Protein Kinetics

Most methods available for measuring interaction kinetics and antibody off-rates challenge an efficient process workflow due to extensive development time and the need for dedicated expert operators. Traditional flow cell methods are hampered by the need for purified samples and their limited capacity for parallel processing.

Assay Principle and Data

Protein binding and dissociation events can be monitored by measuring the binding of one protein in solution to a second protein immobilized on the FortéBio biosensor. The Octet is fully automated and supports multiple binding events on the sensor surface as shown in the workflow below.

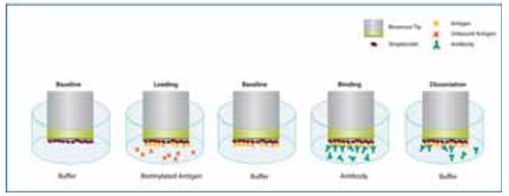

Figure 8. Automated workflow for a model system on the Octet System

Real-time binding kinetic data is presented as depicted in Figure 9.

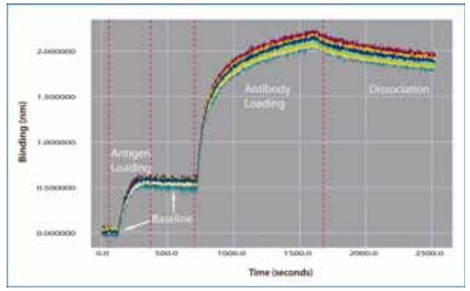

Figure 9. Real-time binding chart of protein binding and dissociation by the Octet System

For methods development, all experimental steps can be automated and monitored on the Octet System. For higher throughput, some steps can be performed external to the instrument, with only critical steps monitored on the Octet. A wide range of approaches can be employed depending on your process requirements.

Reproducibility

To demonstrate reproducibility of protein kinetic results, a series 16 biosensors were assayed in 2 sets of 8, sequentially. The results presented are the calculated kinetics constants of human IgG binding to protein A immobilized on the streptavidin sensor. *(Figure 10)*

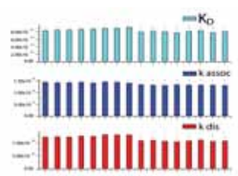

Figure 10. Reproducibility of protein kinetics constants produced from the Octet System

Octet System: Kinetic Screening

In screening mode, crude samples such as periplasmic fractions, analyzed using Octet give the same kd as purified samples. This saves the time and labor that would be required to purify many protein samples (Figure 11).

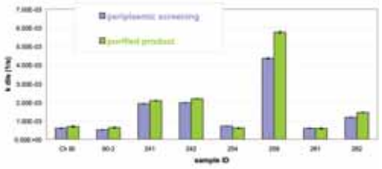

Figure 11. kdis determinations from crude and purified samples

Multi-sampling capabilities

The Octet analyzes samples non-destructively in the microplate well. This means that the sample can be fully recovered or re-assayed. Figure 12 exhibits real-time results from three samples that were assayed in two consecutive runs.

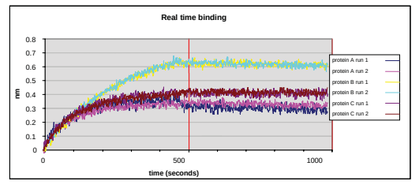

Figure 12. Raw data from duplicate kinetic runs on three proteins

Kd correlation to Biacore

Kinetic data generated using BLI correlate well with Biacore (Figure 13).

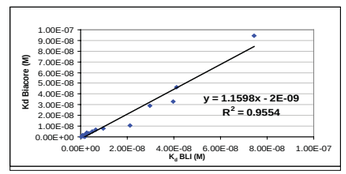

Figure 13. Kd from BLI correlates well with Biacore data

SUMMARY

Octet generates quantitative or kinetic results in real-time, using an integrated system that is user-friendly for training, set-up, assay time and process development. The system provides:

- Real-time results for quantitation and kinetics
- Reporting of kd, ka, kobs, KD
- Minimal interference from media
- Compatibility with crude lysates
- Affinity ranking for 96 samples in 1 hour
- Automation

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