Efficient and cost-effective workflow for antibody bioprocessing using ForteBio's Octet system for label-free, real-time analysis

Krista Witte, Sae Choo, Bettina Heidecker, Jenq-Yuan Yao, Weilei Ma, Robert Zuk, and Hong Tan, ***FortéBio, Inc. Menlo Park, CA***

ABSTRACT

Current analytical methods for antibody bioprocessing require time consuming and technician-dedicated procedures that impact workflow, hands-on time and cost. The Octet - QK generates quantitative or kinetic results in real-time using an integrated system that is user-friendly for training, set-up and assay time.

Octet employs biosensors in a 96-well plate format to report accurate quantitation in 20 minutes for 96 samples, which streamlines the workflow for bioreactors and scale-up columns. In addition, the Octet system can analyze crude media and lysates, simplifying sample preparation and reducing sources of error.

For Kinetic analysis, Octet provides k_{obs}, k_a, k_d and K_D for protein:protein interactions in real time. In screening mode, the dissociation rates for 96 antibodies can be ranked in less than one hour with full analysis and reporting. Octet enables easy characterization of therapeutics during discovery, development and manufacturing stages of bioprocessing.

Octet System for Antibody Process 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.

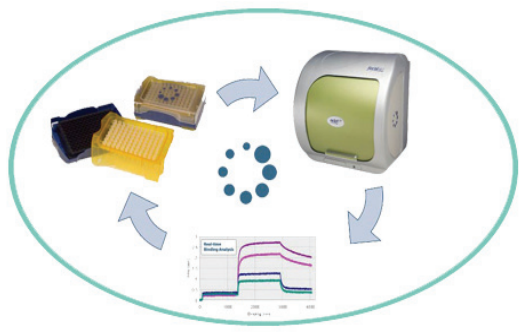


Figure 1. Octet System

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 up to 8 samples in parallel in 7 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.

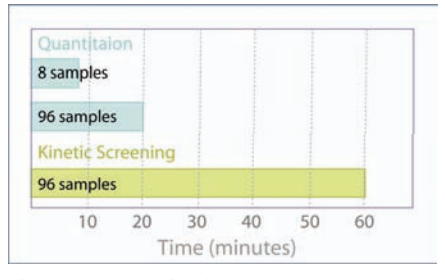


Figure 2. Octet System throughput

Octet System: Quantitation Application and Workflow

Accurate antibody quantitation is critical to the selection of cell lines for development and to optimize production 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).*

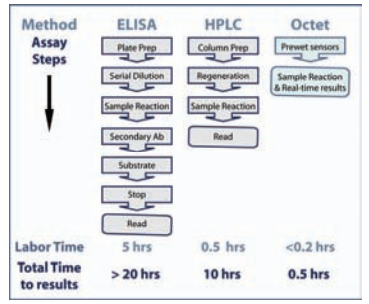


Figure 3. Method comparison for antibody binding

Quantitation Assay Characteristics

Format:	Label-free Optical Biosensors
Plate :	96-well, black plate
Samples:	Crude lysates
Binding Charts:	Real-time
Assay Time:	60 seconds
Sampling:	
- Min.	Up to 8 in parallel
- Max.	96 tests, unattended

Assay Principle and Data

Antibody concentration is determined by measuring the rate of binding to a capture molecule under a set of standard conditions. Binding curves for each standard are generated in real-time *(Figure 4).* Upon completion, a standard curve *(Figure 5)* is automatically derived from the different binding rates and used to calculate the concentration of unknown samples according to user-defined curve fit parameters.

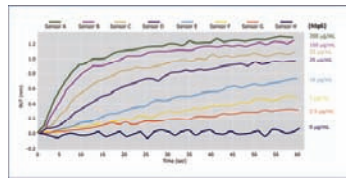


Figure 4. Octet real-time binding curves

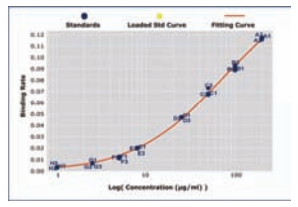


Figure 5. Octet standard curve

Correlation to HPLC

Antibody quantitation from the Octet correlates well with HPLC results.

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

Octet correlation to HPLC is greater than 0.95.

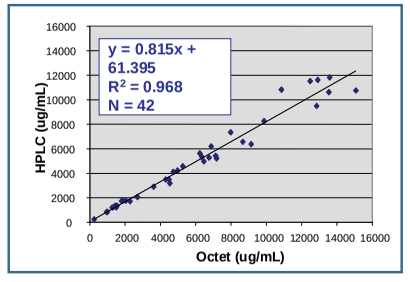


Figure 6. Octet correlation to HPLC

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 *(Figure 7).*

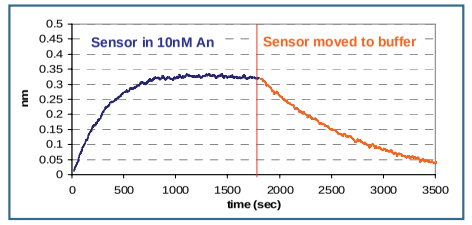
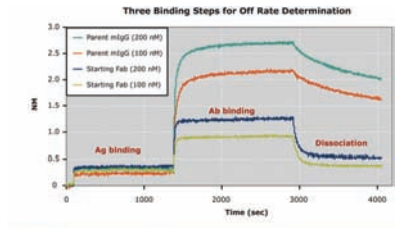


Figure 7. Protein binding and dissociation by the Octet System

Flexible Method Development

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 *(Figure 8).*



Protocol 1	Octet	Octet	Octet
Protocol 2	Off Line	Octet	Octet
Protocol 3	Off Line	Off Line	Octet

Figure 8. Flexibility of Octet method development

Reproducibility

To demonstrate reproducibility of protein kinetic results, a series 8 biosensors were assayed in parallel to monitor the binding of Biotin-Protein A on Streptavidin Biosensors to human IgG *(Figure9).* Octet precision of 3.48 %CV was obtained with excellent residuals values.

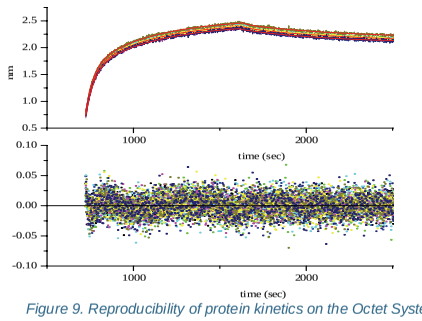


Figure 9. Reproducibility of protein kinetics on the Octet System

Octet System: Kinetic Screening

In screening mode, crude samples such as periplasmic fractions, analyzed using Octet give the same k_d as purified samples. This saves the time and labor that would be required to purify many protein samples (*Figure 10*).

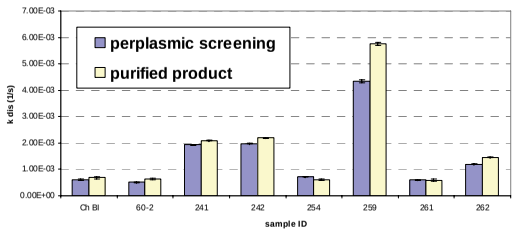


Figure 10. K_d determinations from crude and purified samples

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 11 exhibits real-time results from three samples that were assayed in two consecutive runs.

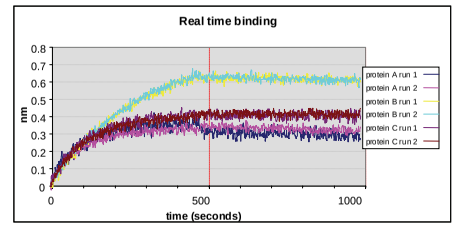


Figure 11. Raw data from duplicate kinetic runs on three proteins

Kd correlation to Biacore

Kinetic data generated using BLI correlate well with Biacore *(Figure 12).*

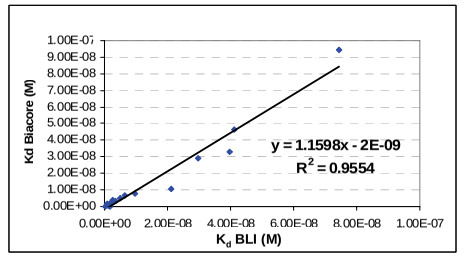


Figure 12. Kd from BLI correlates well with Biacore data

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 K_d, K_a, K_{obs}, K_D
- Minimal interference from media
- Compatibility with crude lysates
- Affinity ranking for 96 samples in 1 hour
- Automation