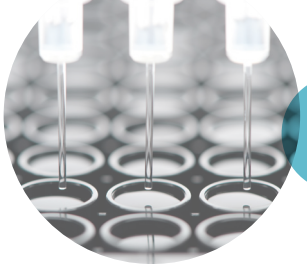
FORTÉBIO
A SARTORIUS BRAND



Octet Systems: Modernize biopharmaceutical QC testing to increase efficiency

Quality control of biological products to support clinical trials and post market assay activities require the evaluation of multiple critical product attributes. The evaluation can include functional assessment for specificity to a target receptor, titer and other quality attributes such as glycosylation, Fc receptor molecule binding, stability assessment to check for the formation of aggregates, etc.

The Octet® platform provides a comprehensive screening and characterization tool across a diverse range of applications during drug development and is especially versatile in antibody and protein quantitation, contaminant testing and general product characterization. This platform circumvents the limitations of ELISA and HPLC platforms, enabling informed decisions to be made earlier in bioprocess development.

Key benefits

- Enhances productivity by increasing capacity to run 20X–40X and 8X–16X more QC potency samples testing/day than ELISA and SPR* respectively
- Automated assay operation allows for 10X less analyst hands-on time than ELISA, resulting in FTE cost savings of > $40,000†.
- Robust instrument resulting in significant maintenance cost savings and low downtime compared to SPR; no fluidics means no clogging of samples and less instrument downtime. You will not need a second backup instrument to support uninterrupted operation.
- Easy to use platform that is approximately 2X faster than ELISA and SPR* in method validation for ligand binding and potency assays

Quantitation assays

The simple Dip and Read™ sample analysis approach that allows for the use of 96- or 384-well plate formats enables streamlined workflows and rapid quantitation of as many as 96 samples in as little as 2 minutes depending on the instrument. In a typical quantitation assay, biosensors coated with capture molecules that bind specifically to the analyte are simply dipped into analyte samples placed in a microtiter plate. The resulting binding is later analyzed using either the rate of the initial slope of binding or the equilibrium of binding; both dependent on sample concentration. To quantify samples, a standard curve is generated using the binding rates of known concentrations of the same analyte as the unknown. The unknown sample concentrations can then be extrapolated from the standard curve.

VALIDATED FAB QUANTITATION ASSAY - BOEHRINGER INGELHEIM (BI)

A key advantage of the Octet instrument's Dip and Read assay format is the ability to rapidly develop methods. The combination of real-time monitoring of assay response with the ability to vary conditions in a sample plate and the instrument's multiple read heads allow for faster assay development when compared to ELISA or SPR. In one such example, the analytical group at Boehringer Ingelheim, Fremont, USA were able to develop an active Fab quantitation assay for in-process testing as well as stability and lot release testing in less than one week. The assay, which required the immobilization of a small molecule onto Streptavidin biosensors resulting in the binding of only active Fab molecules[1], was subsequently qualified for use in QC in less than a month. Compared to their ELISA protocol which required overnight incubation, the Octet Fab assay processed a 96-well

** Compares OctetRED96e and OctetRED384 with a 4-channel SPR system*

† Please contact ForteBio staff for breakdown

A

Theoretical (µg/mL)	Octet assay (µg/mL)	%CV	% Accuracy
2000	682	14.3	34%
1000	702	12.2	70%
500	526	11.3	105%
250	265	2.3	106%
125	128	1.3	102%
62.5	64	2.1	102%
31.3	30	2.7	96%
15.6	13	2.7	83%

B

Theoretical (µg/mL)	Octet assay (µg/mL)	%CV	% Accuracy
400	400.2	3.8	100%
200	200.1	4.2	100%
100	99.9	3.2	100%
50	49.9	3.7	100%
25	25.5	4.2	102%
12.5	12.0	3.8	96%
6.25	5.8	5.9	93%
3.13	3.1	2.6	99%

C

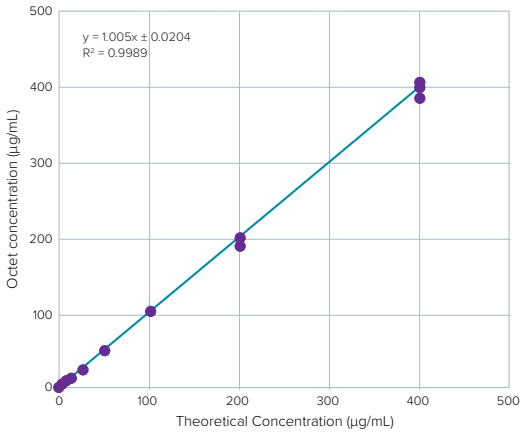


Figure 1: Accuracy assessment for the Fab quantitation evaluated in the range 15.6–2000 µg/mL (A) and 3.13–400 µg/mL (B). Linearity of the Fab assay in the range 3.13–400 µg/mL (C). Data in blue on Figure 1A indicate sample concentrations that fall out of specifications for accuracy or % CV.

plate in one hour, which included sample preparation time. In another study, the Octet system was used as an alternative to HPLC in measuring Fab product concentration in fermentation broth[2]. In these studies, the Octet system was determined to be 20X and 12X faster than HPLC and ELISA respectively with far fewer assay steps (2, 4 and 7 respectively for Octet assays, HPLC and ELISA).

Binding assays

For binding analysis, a ligand molecule can be captured onto the desired biosensor surface based on the chemistry of the biosensor. The choice of the ligand molecule is dependent on the assay of interest; for example, a biotinylated lectin specific to a given glycan could be immobilized onto a Streptavidin biosensor and used to screen for the presence of the glycan on the molecule of interest[3]. A chaperonin protein could also be immobilized in a similar manner and used to screen for the presence of hydrophobic populations in a biological sample to evaluate its stability. Moreover, for antibody:antigen binding assays, off the shelf biosensors including Anti-Human Capture (AHC) and Anti-Mouse Capture (AMC) biosensors allow for the immobilization of antibodies without the need for purification, and are typically cheaper than comparative sensor chips from other label-free technologies.

LEAD MOLECULE CHARACTERIZATION

Once a drug candidate has been purified, functionality studies including receptor binding analysis follow. With three different options: the 8-channel Octet RED96e system, the 16-channel Octet RED384 system and the 96-channel Octet HTX system, the Octet platform is optimally designed to enable the rapid screening of thousands of monoclonal antibody clones for the quick selection of the lead candidate. The technology allows for characterization of effector functions, complement binding and potency assessment amongst other applications through target binding assays. It further facilitates cross blocking studies to enable the grouping of selected clones into bins based on competition for the target antigen's binding epitopes. Further downstream, the Octet system is highly versatile in Fc receptor binding analysis for selected candidates; a variety of off the shelf biosensor chemistries allow for a more flexible assay design.

The interactions of therapeutic antibodies with fragment crystallizable Fc receptors and neonatal Fc receptors (FcRn) are measured in vitro as indicators of antibody functional performance[4]. A primary consideration when developing assays for Fc receptor-IgG kinetic assays is the format. The consideration depends in part on reagent availability. Since Fc receptor-IgG interactions are often relatively low affinity, concentrations of analyte for association may need to be quite high, often in the micromolar range. ForteBio provides users with multiple options for off-the shelf and ready to use biosensor chemis-

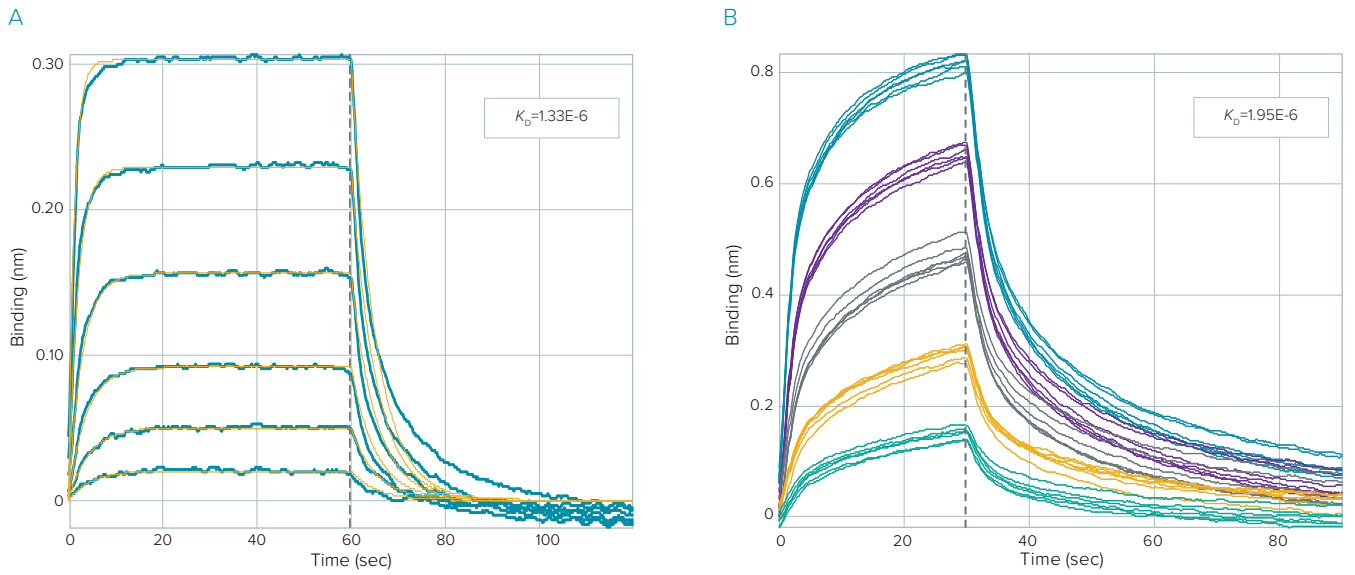


Figure 2: Flexibility in assay design for IgG binding to Fc-gamma IIIa. (A) Anti-Human Fab biosensors used to immobilize the antibody while the receptor molecule is titrated. (B) His-tagged receptor molecule was first immobilized onto Anti-His antibody biosensors followed by a titration of the antibody[5].

tries to fit purpose and circumstances. These include Ni-NTA, antibody-based anti-histidine (Anti-HIS) biosensors (both for his-tagged ligands; commonly available with most vendors of Fc receptor molecules), High Precision Streptavidin biosensors and Anti-Human Fab-CH1 biosensors among others.

It is also important to note that compared to fluidics-based technologies, the Octet platform's design enables it to be better suited for FcRn binding studies where the variable pH conditions required for the different steps of the assay may be difficult to design.

OCTET BIOSENSORS FOR QC ASSAYS

The selection of biosensor surface chemistry depends on the application. All Octet biosensors can be used to develop QC methods. However, during assay method development, repeatability, intermediate precision and reproducibility studies that include biosensor lot to lot assessment should be done to determine assay robustness in line with recommendations from the relevant regulatory bodies. High Precision Streptavidin 2.0 biosensors (SAX2), intended for use with biotinylated ligands, have been developed to ensure minimal lot-to-lot variations in ligand immobilization. This biosensor is recommended for use with any assay where high ligand immobilization reproducibility is critical. The SAX2 biosensor is suitable for both ligand binding kinetics assays and for custom quantitation assays (through pre-coating of a capture ligand on the biosensor).

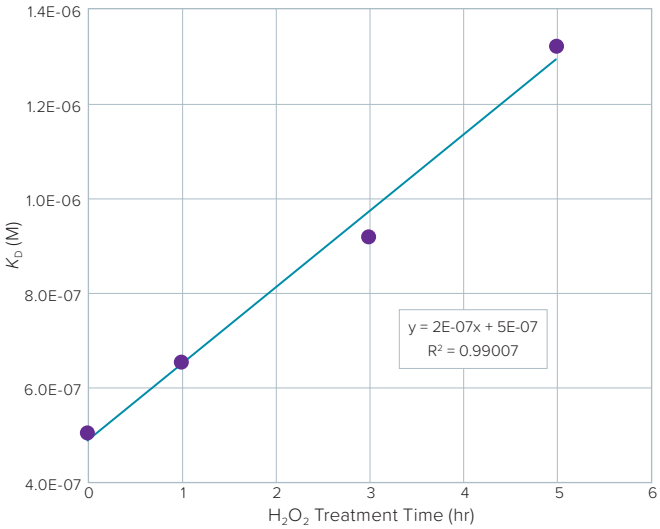


Figure 3: Impact of methionine oxidation on the binding of Herceptin to FcRn. Human IgG1 was incubated in 0.3% H_2O_2 for the indicated 0, 1, 3 and 5 hours respectively before binding kinetics was assessed on an Octet instrument. Data indicates clear inactivation of the human IgG over-time[7].

FORMULATION AND STABILITY ASSESSMENT

Formulation development is the critical last step in downstream bioprocessing. Production and storage media play a critical role in the activity and stability of biological therapeutic drugs. Unlike traditional tools like dynamic light scattering (DLS) and Circular Dichroism (CD) that mainly measure stability parameters such as onset of aggregation or melting temperatures, the Octet platform can be used to evaluate both stability and functional characteristics of these biological molecules. A group at Kansas University Medical Center (KUMC) developed an automated method using biotinylated GroEL-streptavidin biosensors with Bio-Layer Interferometry (GroEL-BLI) to detect the formation of transiently formed, pre-aggregate species in various pharmaceutically relevant monoclonal antibody (mAb) samples. The relative aggregation propensity of various IgG1 and IgG4 mAbs was rank ordered using the GroEL-BLI biosensor method, and the least stable IgG4 mAb was subjected to different stresses including elevated temperatures, acidic pH, and addition of guanidine HCl[6]. In another study, the Octet platform was used to rapidly evaluate the effect of oxidation on the functional activity of Herceptin through the binding characterization of the drug product against an FcRn molecule. In this study, the Dip and Read plate format allows for the incubation of the drug product in peroxide (H_2O_2)in the sample plate in a time-staggered manner followed by the analysis of drug activity at these various time points (Figure 3).

GxP-ready solutions

Octet platforms come with a complete GxP package that include instrument installation and qualification (IQOQ) kits, performance qualification (PQ) kits and 21 CFR Part 11-compliant software. In addition, ForteBio provides users with software and biosensor validation support that facilitates compliance with GMP requirements.

References

1 ForteBio Application Note 12: Validated Quantitation and Activity Assay of Antibody Fragment Molecule (Fab).

2 LCGC North America: Biolayer Interferometry as an Alternative to HPLC for measuring Product Concentration in Fermentation Broth; Anurag S. Rathore, Deepak Kumar, Jyoti Batra, Ira Krull

3 Biotechnol Prog : Lectin bio-layer interferometry for assessing product quality of Fc- glycosylated immunoglobulin G Wallner J, Sissolak B, Sommeregger W, Lingg N, Striedner G, Vorauer-Uhl K, 2019 35(5):e2864. doi: 10.1002/btpr.2864.

4 Febs Open Bio: Rapid screening of IgG quality attributes – effects on Fc receptor binding; Karin P. M. Geuijen, Cindy Oppers-Tiemissen, David F. Egging , Peter J. Simons, Louis Boon , Richard B. M. Schasfoort and Michel H. M. Eppink, 2017 https://doi.org/10.1002/2211-5463.12283

5 ForteBio Application note 17: Analysis of Fc-gamma Receptor-IgG interactions on the Octet platform.

6 The Use of a GroEL-BLI Biosensor to Rapidly Assess Pre-aggregate Populations for Antibody Solutions Exhibiting Different Stability Profiles; Samantha E. Pace, Sangeeta B. Joshi, Reza Esfandiary, Robert Stadelman, Steven M. Bishop, C.R. Middaugh, Mark T. Fisher, David B. Volkin, *J Pharm Sci.* 2018 107(2):559-570. doi: 10.1016/j.xphs.2017.10.010.

7 Sangeeta B. Joshi, Reza Esfandiary, Robert Stadelman, Steven M. Bishop, C.R. Middaugh, Mark T. Fisher, David B. Volkin, *J Pharm Sci.* 2018 107(2):559-570. doi: 10.1016/j.xphs.2017.10.010.

FORTÉBIO
A SARTORIUS BRAND

FB_4021 Rev B

FOR SALES & SERVICE VISIT:
fortebio.com/contact-us
+1 650 322 1360 | info@fortebio.com