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Octet[®] Systems: Modernize Biopharmaceutical QC Testing to Increase Efficiency

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Abstract

The Octet[®] BLI instrument is a sample plate-based platform with no micro-fluidics and is designed to enhance productivity and to save costs for users in the quality control laboratories. The easy to use platform is a robust platform with minimal down time and allows for enhanced productivity through increased capacity due to its high throughput capabilities and minimal analyst involvement. The Dip and Read assay format allows for the ability to rapidly develop methods that span applications across the drug development workflow. 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 lead molecule characterization, quantitation and other ligand binding assays method development and makes the technique more suitable for quality control applications than other commonly used techniques such as ELISA and SPR.

In addition, the availability of off-the shelf and ready to use biosensors together with GxP-compliant products such as 21 CFR Part 11 software, Installation and Operational Qualification (IQOQ) and Performance Qualification (PQ) kits means that Octet[®] users in quality control labs can perform lot release and in-process testing applications with confidence.

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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 molecules¹, 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 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 broth². 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).

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

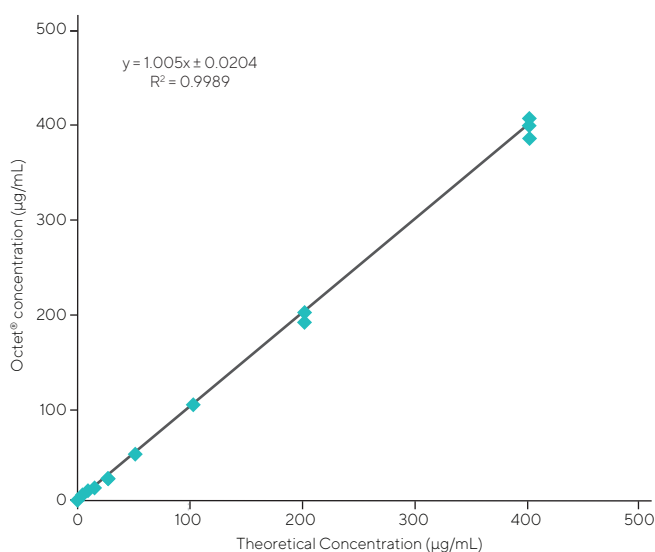


Figure 1: Accuracy assessment for the Fab quantitation evaluated in the range 15.6–2000 µg/mL (A) and 3.13–400 µg/mL (B). Linearity of the Fab assay in the range 3.13–400 µg/mL (C). Data in bold on Figure 1A indicate sample concentrations that fall out of specifications for accuracy or % CV.

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 interest³. 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 Fc receptors and neonatal Fc receptors (FcRn) are measured *in vitro* as indicators of antibody functional performance⁴. 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

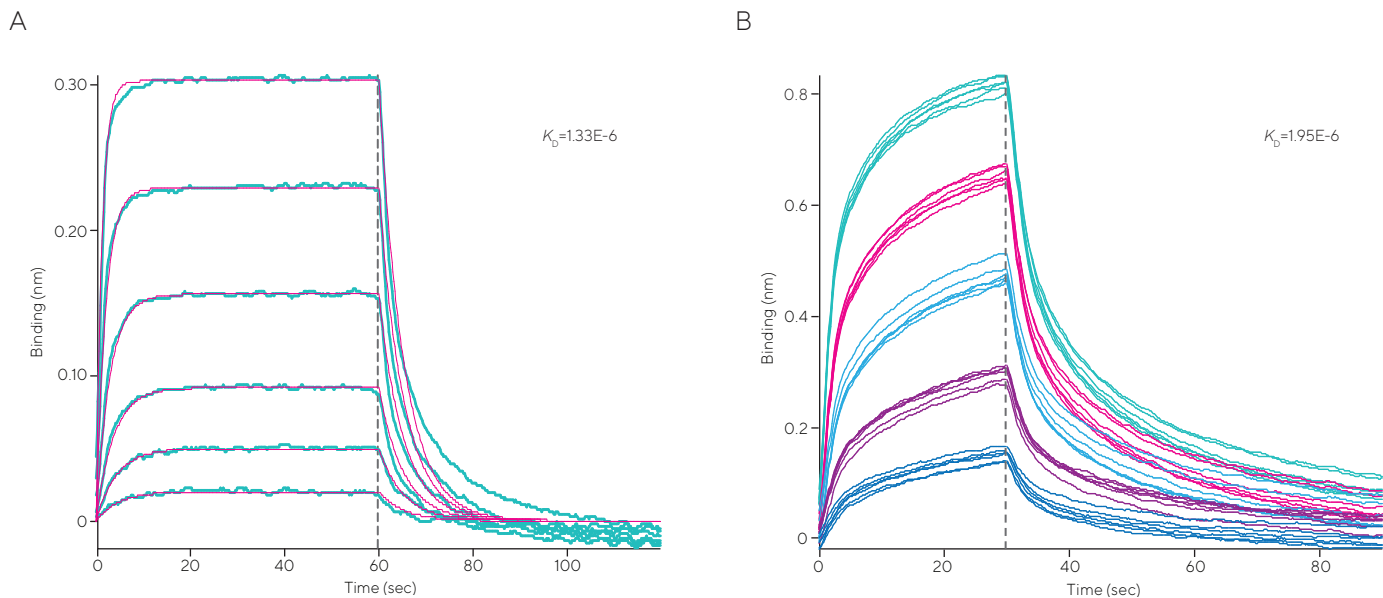


Figure 2: Flexibility in assay design for IgG binding to Fc-gamma IIIa. (A) Anti-Human Fab Biosensors used to immobilize the antibody while the receptor molecule is titrated. (B) His-tagged receptor molecule was first immobilized onto Anti-His antibody Biosensors followed by a titration of the antibody⁵.

analyte for association may need to be quite high, often in the micromolar range. Sartorius provides users with multiple options for off-the-shelf and ready-to-use biosensor chemistries 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).

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 HCl⁶. 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 hydrogen peroxide (H₂O₂) in the sample plate in a time-staggered manner followed by the analysis of drug activity at these various time points (Figure 3).

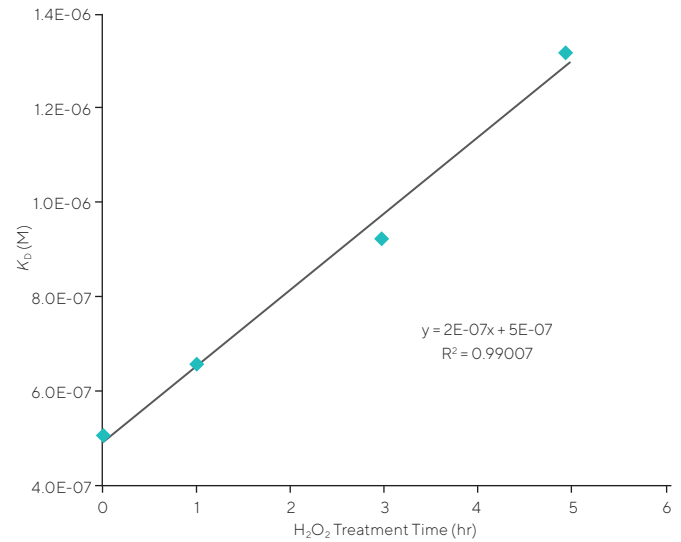


Figure 3: Impact of methionine oxidation on the binding of Herceptin to FcRn. Human IgG1 was incubated in 0.3% H₂O₂ for the indicated 0, 1, 3 and 5 hours respectively before binding kinetics was assessed on an Octet® instrument. Data indicates clear inactivation of the human IgG over-time⁷.

GxP-Ready Solutions

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

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