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Enhanced Productivity and Labor Efficiency in Lot Release and In-Process Testing of Biologics in GxP Laboratories

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Abstract

Octet® systems come with distinct advantages including ease of use, throughput, low maintenance, and microfluids-free configurations, enabling them to provide GxP users with enhanced productivity and labor efficiency for lot release and in-process testing of Biologics. Octet® instruments can be equipped with Octet® 21 CFR Part 11 Software for compliance with regulatory requirements. Compliance is further enhanced with the availability of instrument qualification kits including IQOQ and PQ kits that ensure the platform performs as stipulated. This whitepaper includes a few examples in which Octet® systems have been used under GxP compliance for different applications and shows the relative benefits over alternate technologies.

Key Considerations

- **Enhanced productivity:** Enhances productivity by increasing the capacity to run 20X–40X and 8X–16X more QC potency samples testing/day than ELISA and SPR* respectively.
- **Cost savings:** Reduced hands-on operation allows for 10X less analyst hands-on time than ELISA, resulting in FTE cost savings of > $40,000*.
- **Time savings:** 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.
- **Ease of use:** The Octet® platform is approximately 2X faster than ELISA and SPR* in method validation for ligand binding and potency assays.
- **Complete Octet® GxP package:** Comes with all requirements for GMP compliance – IQOQ protocols and kits, user guides, performance qualification (PQ) protocols and kits, Octet® 21 CFR Part 11 Software with audit trails, software validation package and biosensor validation support. Octet® R8 and Octet® RH16 systems come with specifically optimized performance qualification kits.
- **Successful use:** Currently validated by and used in multiple CROs and CDMOs world-wide.

* Please contact Sartorius for details.

Introduction

Bio-Layer Interferometry (BLI) is an optical technique that analyzes the interference pattern of white light reflected from two surfaces: a layer of immobilized protein on the biosensor tip and an internal reference layer (Figure 1A). Any change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern that can be measured in real time (Figure 1A and 1B). The binding between a ligand immobilized on the biosensor surface and an analyte in solution produces an increase in optical thickness measured as a wavelength shift, Δ λ (Figure 1C).

Sartorius' Octet® instruments utilize BLI technology to monitor biomolecular interactions in real time. They are an ideal replacement for ELISA and HPLC techniques for the quantification of antibodies and recombinant proteins and are especially suitable for product potency lot release assays. The platebased and non-fluidic format also offers GxP users distinct advantages over comparative SPR based techniques. Octet® systems provide higher throughput, with the flexibility to run 2 to 96 samples simultaneously, and better sample versatility, including the ability to analyze crude samples and more tolerance to diverse sample matrices. In addition, ease of use, low maintenance and high data precision speeds time to results throughout the drug development process. The Octet® platform is particularly well-suited for GxP and QC laboratories.

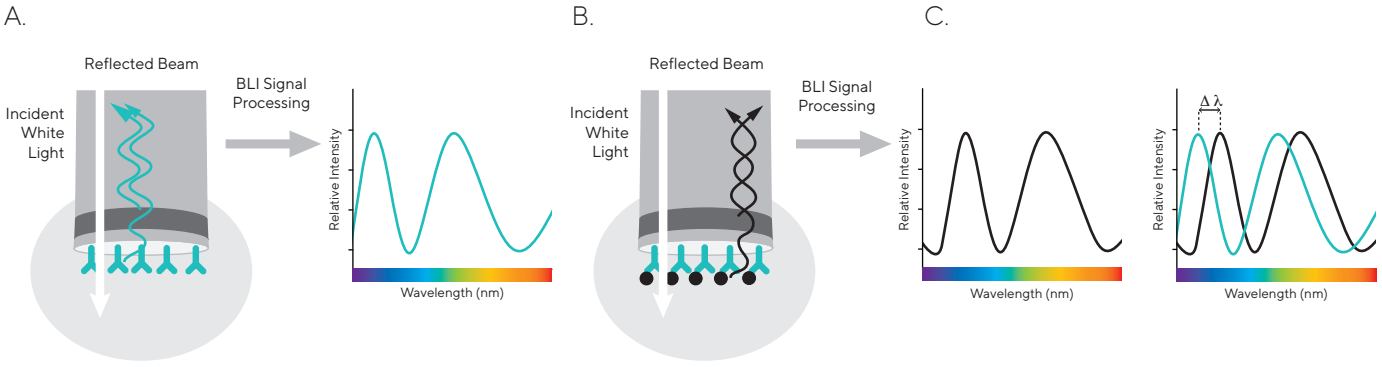

Figure 1: Relative intensity of the light reflection pattern from the two surfaces on the biosensor. Octet® sytems with BLI technology measure the difference in reflected light's wavelength (Δλ) between the two surfaces.

Figure 2: Sartorius' Octet® systems meet a wide range of throughput needs. The higher throughput Octet® RH16 and RH96 systems analyze either 16 or 96 samples at a time, respectively (top). The lower throughput Octet® R Series systems analyze either 2, 4 or 8 samples at the same time respectively (bottom).

Analytical Instruments in the GxP Environment

Analytical methods that provide reliable and accurate data are necessary to ensure quality standards are met during the production and release of drug products. In order to ensure data accuracy, it is critical that the performance of the applied analytical instruments, the computer systems used, and the developed methods for data acquisition meet required specifications.

Several official regulatory guidelines including Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP) and ISO Standards exist and provide guidance on meeting compliance.

Sartorius, an ISO 9001 certified company, offers Octet® 21 CFR Part 11 Software and full GxP products and services for Octet® instruments (Figure 2). These include:

- Octet® CFR Software and Sartorius FB server features for secure, traceable electronic record keeping that enable compliant data acquisition and data analysis in laboratories working under GxP and 21 CFR Part 11 regulations.
- IQOQ/PQ packages that ensure that Octet® systems are qualified and operate as intended and that the performance meets specifications.
- Customer-run software validation support
- Biosensor validation support services
- Technical support assistance

Octet® Instrument Qualification

Instrument qualification is critical to meeting compliance guidelines in any analytical laboratory developing, qualifying and validating assays in the GxP environment. Sartorius offers a rigorous installation qualification and operational qualificaton (IQOQ) kit that includes a user guide, reagents, and documentation required for instrument installation and qualification. In addition, a comprehensive performance qualification (PQ) kit is also available that can be used for performance qualification and periodic system checks. The IQOQ and PQ kits can be purchased separately at the time of instrument installation or any other time for regular system performance checks to ensure calibration and maintenance of the instrument.

Installation Qualification and Operational Qualification (IQOQ)

The IQOQ Kit provides a checklist of instrument components and documented verification that the Octet® instrument, accessories and computer system as supplied and installed, comply with Sartorius' specifications. It also provides a comprehensive and documented verification that the Octet® instrument operates as intended. It verifies that various critical operational parameters such as the optical system, alignment of instrument, plate, temperature and sample plate shake speed meet the stipulated specifications. The IQOQ Kit can be added at time of instrument purchase or at any other time during the lifespan of the Octet® instrument. Sartorius recommends the IQOQ procedure to be repeated after an instrument move or repair and following a software upgrade.

Performance Qualification (PQ)

The PQ Kit is used to verify that the Octet® instrument is fully functional after installation. System functionality is tested for both quantitation and kinetics analysis. The kit contains biological samples, reagents and biosensors for each analyses and is used to demonstrate that consistent and reproducible results are obtained within product specifications.

The qualification process for kinetics applications is two-fold:

1. Assess instrument performance for ligand loading onto the biosensor surface with stipulated loading acceptance criteria
2. Assess instrument performance for analyte binding to the immobilized ligand with stipulated acceptance criteria.

The qualification process for quantitation applications asseses performance of known concentrations of Prostate Specific Antigen (PSA) used in the generation of a standard reference curve. Assay performance is evaluated against stipulated percent acceptance variation statistics.

Octet® CFR Software and Software Validation Services

Software validation and the use of 21 CFR Part 11-compliant software are regulatory requirements prior to using any analytical instruments in a GxP environment. Octet® CFR Software includes features such as controlled user access and electronic signatures. In addition, the Sartorius FB Server module manages the information recorded during user sessions. Software validation provides users with the confidence that data integrity and accuracy are always maintained. The Software Validation services provide a single source for meeting all software compliance needs, including demonstration that the Octet® Software is compliant with the 21 CFR part 11 final rule. This validation package includes an instruction manual that provides guidelines on how to verify functions in the FB Server monitor and Octet® Analysis Studio Software. In addition, it provides example data sets for the comparison of Octet® software-generated data to other software such as Microsoft® Excel® and GRAPHPAD PRISM™.

High-Precision Biomolecular Analysis

BLI is a widely adopted technique in biopharmaceutical development and manufacturing. The throughput and flexibility of the Octet® platform has helped accelerate almost every stage of the drug development workflow (Figure 3) in leading and start-up biotherapeutic companies, enabling informed decisions to be made earlier.

The comprehensive tool set for compliance make Octet® systems ideal in regulated quality control (QC) labs for concentration and impurity analysis in both upstream and downstream processes. In addition, Octet® systems have become a solution platform for kinetic and potency analysis of drug-target and drug-Fc receptor(s) (FcγRI, FcγRIIa, FcRIII and FcRn) interactions, and for stability analysis by assessing changes in activity in stressed and forced degradation samples. The high-throughput capability and flexibility of the platform enables rapid completion of assay method development through design of experiments (DOE) essential in identifying and controlling critical quality attributes (CQAs) that ensure consistent and reliable quality of biopharmaceutical products.

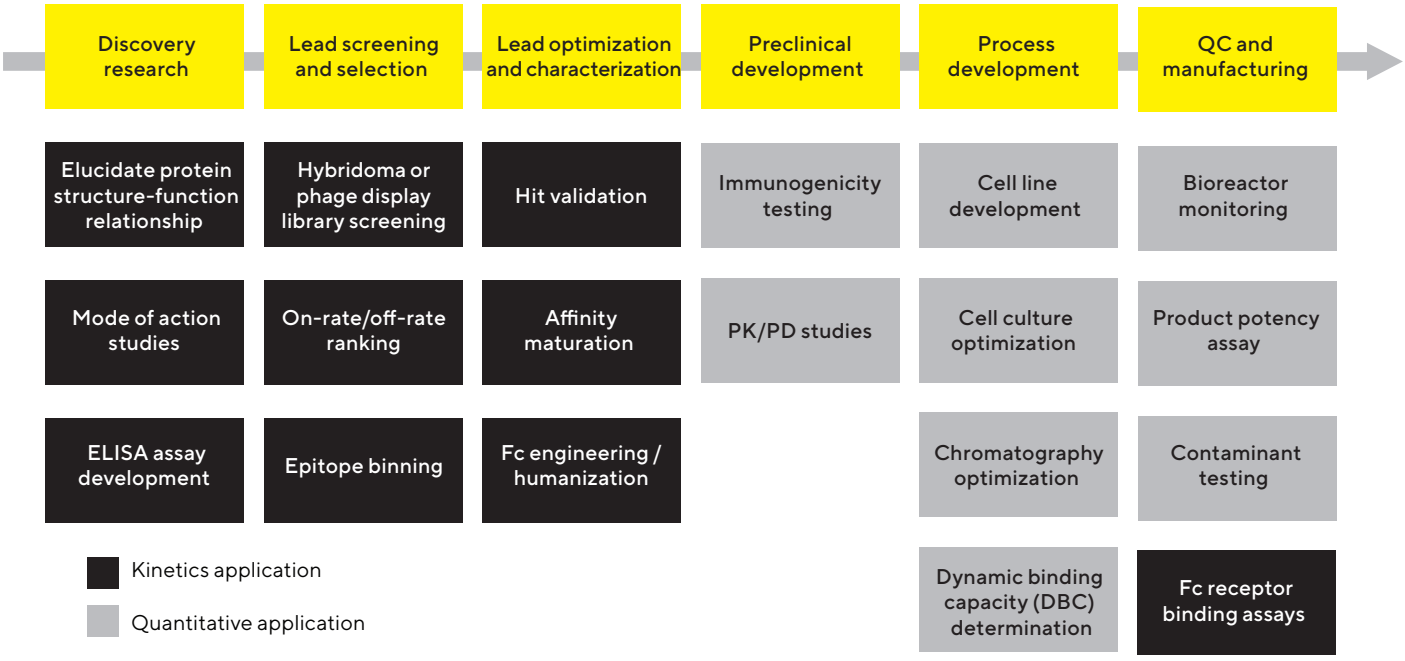

Figure 3: Select applications areas of Octet® systems in biotherapeutics development.

Biosensor Validation Services

Sartorius provides GxP users with biosensor support during the method validation process. Customers are able to order multiple lots of a biosensor for assay method qualification and validation, and can reserve one of the lots for future purchase.

The service is available for the following biosensor types: Protein A (ProA), Streptavidin (SA), High Precision Streptavidin (SAX), Ni-NTA (NTA) and Anti-Mouse Capture (AMC). Customers can order up to five different biosensor lots, up to 20 trays from each lot for evaluation, and reserve up to 40 additional trays for future purchase.

High Precision Streptavidin 2.0 (SAX2) Biosensors

Sartorius provides GxP users with High Precision Streptavidin 2.0 (SAX2) Biosensors developed and qualified for applications in downstream drug discovery and regulated environments that have stringent assay precision requirements. SAX2 is QC-tested for the biotinylated molecule to meet precisioncontrolled coefficient of variation (CV) specification of ≤ 4% within lot and CV range of 20% across lots. SAX2 is suitable for both kinetics and quantitation assays when used with biotinylated ligands.

Concentration Determination

The Octet® platform provides a convenient and reliable analytical method for measuring antibody and protein concentrations. The simple Dip and Read approach enables streamlined workflows and rapid quantitation of 96 samples in as little as 5 minutes on the Octet® RH96 system or 80 samples in < 30 minutes for the Octet® R8 system. Samples can be analyzed in cell culture supernatant or in complex media, thus removing the need for purification.

In a typical quantitation assay, biosensors coated with capture molecules are dipped into samples in the sample plate and the on-rate is measured in real time. The measured on-rate is then used to determine the concentration of the target protein. This is done by comparing the binding signals obtained from a set of known analyte concentrations which are used to generate a standard curve with the signals from the unknown samples. Commonly used biosensors for antibody and protein concentration determination include Protein A- and Protein G-coated Biosensors and Anti-Human IgG (AHQ) and Anti-Mouse IgG (AMQ) Biosensors for antibody or Fc based protein molecules. Anti-HIS (HIS2), Anti-GST (GST) and High Precision Streptavidin Biosensors (SAX) are used for quantitation of recombinant proteins. Octet® instruments measure the unique binding properties of interacting pairs of molecules; hence the calibration with external standards used with techniques like OD 280 measurements are not needed. The instrument's performance is verified through well-characterized materials that can be used to confirm the system is running within set specifications. Since the binding properties of each specific interaction pair is unique, a stan dard or reference curve using the purified version of the analyte in matching matrix is required. As the association curve of a binding event is used to quantify the analyte in the sample, only the amount of biomolecule that really binds to the immobilized ligand on the biosensor is taken into consideration.

In quantitation assays, precision, linearity and accuracy are key parameters that must be demonstrated in order for the assay to be validated for use in manufacturing. In addition, the limit of quantitation (LOQ) should be determined to increase confidence in the assay's performance. As an example, the Octet® system was used to determine method precision and accuracy in the quantitation of recombinant insulin.[1] High Precision Streptavidin Biosensors (SAX) were coated with anti-insulin antibody and used to bind purified insulin samples. Known insulin sample concentrations were prepared in Sartorius' 1X Kinetic Buffer at concentrations ranging from 0–50 µg/mL and used in binding assays (Figure 4A) to generate a standard/reference curve (Figure 4B). Three samples (25, 6.25 and 1.56 µg/mL) of insulin were also spiked in 1X Kinetics Buffer and treated as unknown samples. All samples were run in triplicate. Data was analyzed using the initial slope binding rate analysis mode in Octet® Analysis Studio Software. The standard samples exhibited concentration % CVs of < 3% (Table 1), while the unknown samples exhibited concentration % CVs well below 5% indicating excellent precision. Dose recovery for the unknown samples was found to be within 90–110%, indicating excellent method accuracy (Table 2). A similar approach can be used for other recombinant proteins.

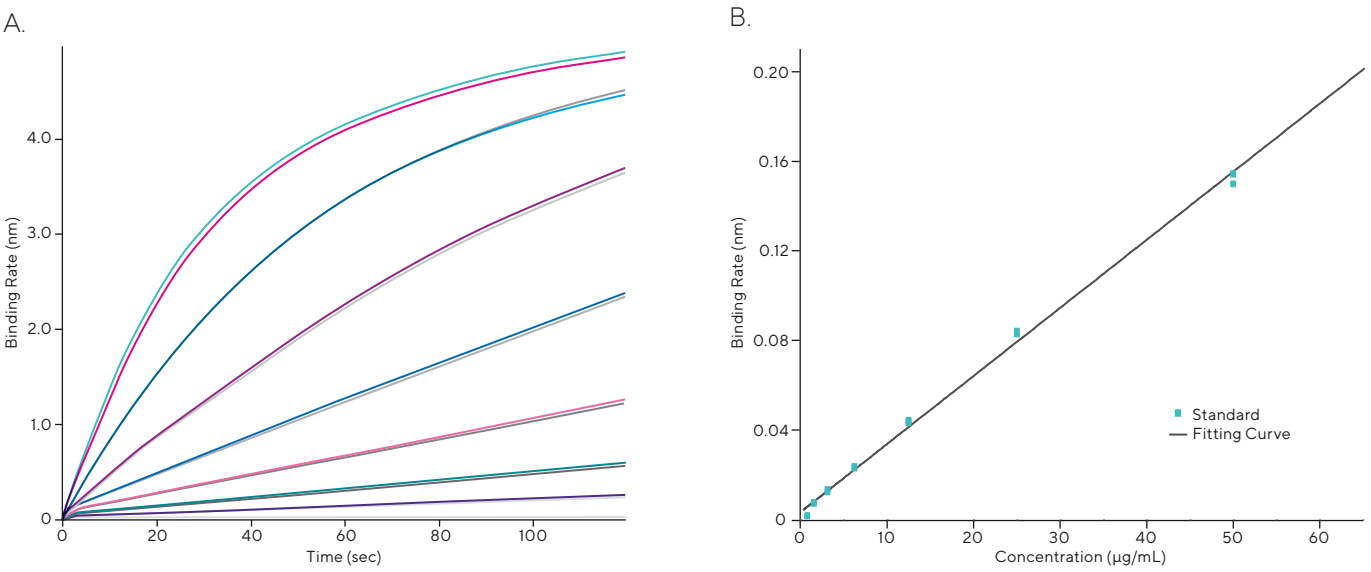

Figure 4: Binding of known concentrations of recombinant insulin (A) used to generate a standard/reference curve (B) for the quantitation of recombinant insulin. SAX Biosensors loaded with anti-insulin were used for the studies.

Table 1: Insulin quantitation. Standard samples exhibit excellent precision at < 3% CV.

Conc. µg/mL	Binding rate, nm/s	Conc. avg, µg/mL	Conc. % CV
50	1.02	50	2.39
25	0.5	25	1.88
12.5	0.1872	12.5	1.24
6.25	0.0611	6.2	1.48
3.13	0.0189	3.1	0.3476
1.56	0.0058	1.6	2.83
0.78	0.0012	0.8	0.2831
0	0.0001	0	0

Table 2: Insulin quantitation. Unknown samples, n=3, exhibit excellent precision and accuracy.

Conc. µg/mL	Binding rate, nm/s	Conc. avg, µg/mL	Conc. % CV	% Recovery
25	0.5182	25.9	1.41	104
6.25	0.064	6.4	0.1638	102
1.56	0.0066	1.7	1.4	106

High-precision Ligand Binding and Potency Assays

Ligand binding kinetics assays are increasingly finding use as batch lot release methods; especially in potency assays where an assessment of product stability and function can be made. In these binding kinetics studies, the interaction is often assessed through the measurement of either the affinity of the analyte/drug product to a receptor or ligand immobilized on the bisensor surface or by monitoring the drug product's binding response signals (Req) as a function of concentration and comparing it to a control reference product for relative potency assesments[2]. Sartorius offers ready-to-use biosensors such as Ni-NTA and FAB2G Biosensors for the immobilization of different panels of Fc gamma receptors that can in turn be used to bind drug products for potency assesment.[3]

In all cases, reproducibility measured through precision and accuracy are key metrics. Octet® systems are highly suitable for these assays as they decrease method development time significantly compared to SPR and ELISA techniques. An overlay of replicate data (Figure 5) for the binding of an FcRn molecule to an IgG, with the IgG immobilized onto Anti-Human Fab-CH1 (FAB2G) Biosensors shows excellent reproducibility.

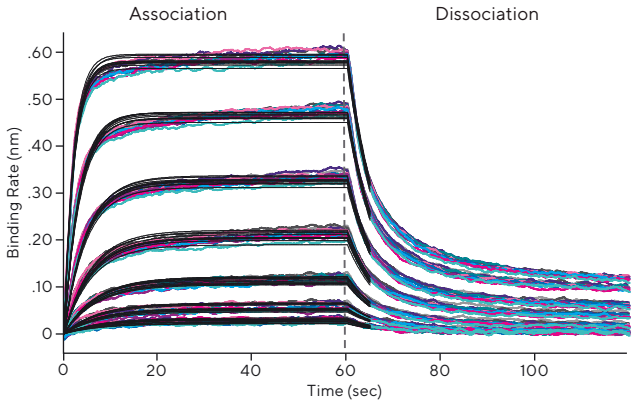

Figure 5: Overlay of several replicates of FcRn-IgG interactions on the Octet® platform using FAB2G Biosensors. Fitting of data traces was done using a 1:1 model with global fitting and a 5-second dissociation step (fit lines in magenta).

Stability and Forced Degradation Studies

Octet® platforms can also be used for developing stability-indicating methods, and are suitable for measuring and distinguishing between fully-functional drug products and those whose binding activities have been affected by degradation. In one study, the Octet® platform was used to distinguish between native and deactivated antigen and showed reduced binding activity of the deactivated antigen (ref), hence proving the assay was stability indicating.
In another study[4] (Figure 6), the affinity of an IgG1 to an Fc gamma receptor IIIa molecule was shown to decrease with increasing percent deglycosylation, further indicating that Octet® systems are suitable for use in developing stability-indicating methods.

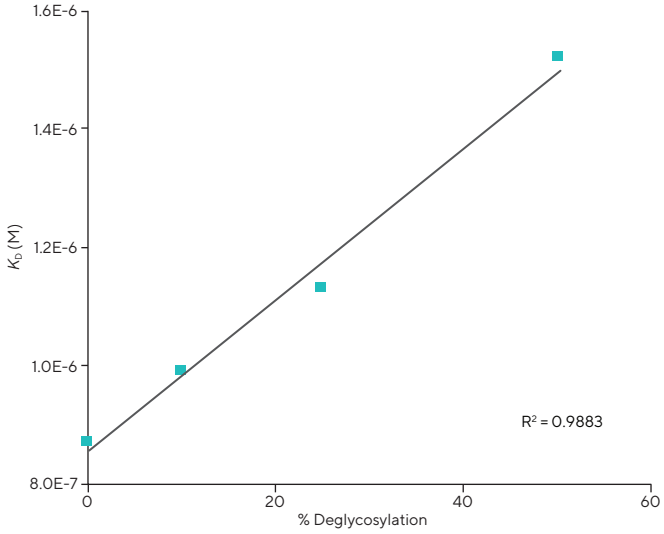

Figure 6: Affinity analysis of the binding of an IgG1 to an Fc gamma receptor as a function of deglycosylation.

Technical Support

Sartorius offers comprehensive technical and service support for Octet® system users in the GxP environment. Field Applications Scientists and Engineers are trained to provide guidance in various aspects of compliance, including assay development and qualification. Performance certification (PC) that includes instrument preventive maintenance and the necessary documentation is also available to GxP users, and is recommended every 6 months.

Summary

Sartorius offers Octet® system users complete qualfication, validation and support solutions that ensure compliance in the regulatory space, and allow rapid development, optimization and validation of assay methods for various applications in GLP and QC laboratories. A summary of product offerings and assay recommendations are listed in Table 3.

References

1. Steve Turner et al., Sartorius Application Note: Customized Quantitation of Recombinant Therapeutic Proteins Using High Precison Streptavidin (SAX) Biosensors.
2. Nathan Oien et al., KBI Potency webinar: From Screening to QC: Development considerations for Octet® methods.
3. Michael Sadik et al., Bioprocessing Online, Accelerate Biopharmaceutical Development with Novel Analytical Techniques, April 28th, 2017.
4. Renee Tobias et al; Sartorius Application Note: Analysis of FcRn-Antibody Interaction on the Octet® Platform.

Table 3: Sartorius Octet® platform GxP product offerings and typical GxP/QC applications.

Item	Description
IQOQ Kit and service	Includes items required for the verification and documentation of instrument components and for the qualifcation of instrument operational features
PQ Kit	Includes biosensors and reagents required to verify that the instrument performance meets specifications in binding and quantitation applications
Octet® CFR Software	21 CFR Part 11 data acquisition and analysis software that ensures compliance with 21 CFR Part 11 regulations
Software validation support	Sartorius offers user support during validation of Octet® Software
Biosensors for validation support	Sartorius will work with customers to sequester multiple lots of biosensors (for a select group of biosensors) during assay method qualification and method validation processes
Key recommended Octet® platform GxP applications	Lot release, in-process testing and investigational new drug (IND) testing assays, potency assays, ligand binding, stability, detection limit and titer determination methods

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