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Application Guide

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Octet® Systems in Bioprocessing: Easy-to-Use and Cost-Effective Tools for Multiple Applications

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Abstract

Optimization of upstream processes and cell culture conditions are leading to increased production yields of biologics. Along with these improvements, advances in analytical technologies are also required to facilitate titer determination and assessment of critical quality attributes early in the discovery process. This application guide describes the Octet® platform, biosensors, and assay kits that offers intermediate and high throughput capabilities for titer, host cell protein analysis, residual protein A detection and sialic acid content detection and the associated time and cost savings.

Advances in the optimization of upstream bioprocessing in recent years - primarily, improved cell culturing conditions - have led to higher production of target biologics. This leads to amplified production of process related attributes as well[1]. The selection and optimization of bioprocessing therefore, requires the integration of analytical technologies that facilitate both titer determination as well as critical quality attributes assessment of these biologics early on in the discovery and optimization stages. One platform that is an industry accepted workhorse for multiple analytical applications in bioprocessing is the Octet® platform. The Octet® instrument comes with biosensors and assay kits that offers users both intermediate and high throughput capabilities for titer, host cell protein analysis, residual protein detection and sialic acid content detection. The fluidics-free system allows users to screen for these properties without the need for purification, resulting in significant time and cost savings; it is estimated that with the Octet® platform, as much as 12X FTE costs can be saved when compared to ELISA in IgG titer. In addition, the Octet® RH16 and RH96 systems are automation-ready, allowing for extended walk-away assay times.

- Automation ready platforms suitable for multiple applications (Octet® RH16 and RH96 systems)
- Real time label-free data acquisition enabling rapid assay optimization
- Sample plate format allowing for the use of crude and non-purified samples
- Combine titer and sialic acid analysis from the same sample. Analyze 1000 samples in one day on the Octet® RH96 system
- Complete hands-off, walk-away HCP analysis on the Octet® RH96 system
- High precision assays with 5–10% CVs
- Detection sensitivity as low as 0.5 ng/mL for HCP assays and 0.1 ng/ml for residual Protein A
- No heating or centrifugation steps required for residual Protein A analysis

Titer and Glycan Screening

The Octet® platform is routinely used for titer determination, especially with monoclonal antibodies in both upstream and downstream bioprocessing. The Octet® RH96 instrument is capable of analyzing as many as 96-samples in just two minutes in a simple Dip and Read format, where biosensors pre-coated with Protein A or G or other antibody binding proteins are dipped into IgG samples for specific binding response measurement. In addition to titer, the Octet® platform is also compatible for use in glycan screening. For example several groups used this method for different glycans screening[2]. A common approach for the screening of glycans on the Octet® system involves the immobilization of sugar-specific lectins onto the biosensor surface followed by dipping the coated biosensor into the sample under certain buffer conditions. Sartorius has recently released a kit (Part No. 18-5135) for the screening of sialic acid content in biologics which can be used in combination with titer determination to determine sialic content per mg of IgG Figure 1.

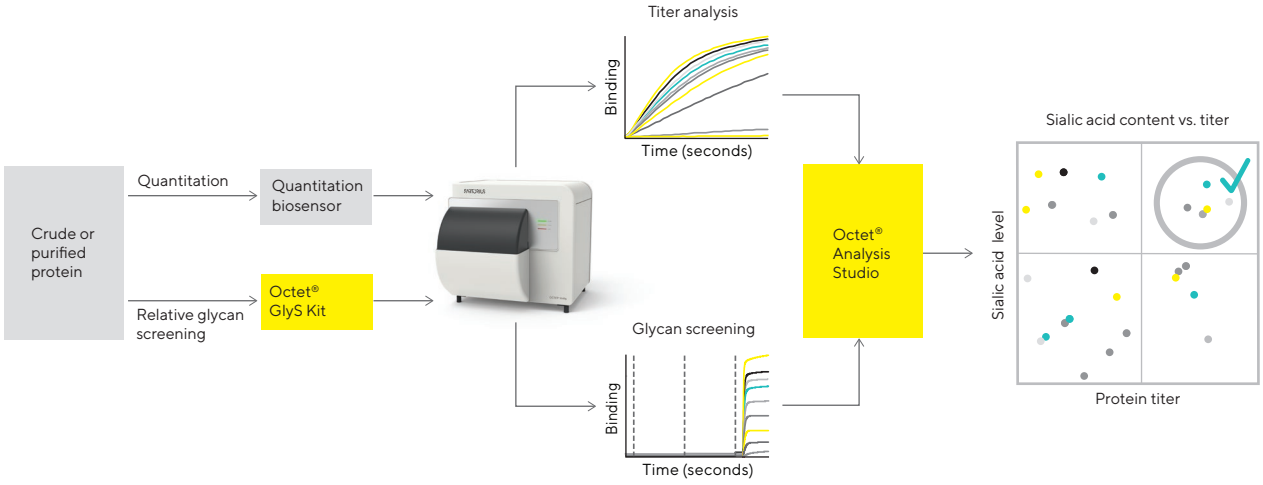

Figure 1: Titer and sialic acid work-flow on the Octet® system. Sialic acid versus normalized titer can be used to select the best conditions for bioprocessing.

Residual Contaminant Detection

Contaminants are any molecules that may elute with the target drug product during purification. They can adversely affect the efficacy and immunoreactivity of the drug product and should therefore be cleared from the product through further purifications. The easy to use BLI technology has comparable throughput to manual ELISA but with better precision in contaminant detection. In addition, the platform shows data in real time allowing for a rapid optimization of assays.

Transfer Your ELISA Host Cell Protein (HCP) Detection Assay to the Octet® Platform

The clearance of host cell proteins (HCPs) that co-express with biologics is important since high concentrations can adversely affect the safety and efficacy of the biologics. Sartorius' HCP kit comes with all the reagents required to convert a manual HCP ELISA assay into a better controlled automated assay with lower variability (Table 1) and where data can be observed in real time. Unlike ELISA, real time analysis techniques allow assay developers to monitor every step of the assay enabling the fast detection of areas that need further optimization. The kit comes with biosensors already coated with a Cygnus capture antibody, a purified antigen for the development of the reference curve and the detection reagents (Figure 2). The Octet® RH96 system can be used to screen > 1000 samples in one day making it highly suitable for screening for these process impurities in a high throughput manner.

Assay performance	Cygnus 3G ELISA Kit	Sartorius-Cygnus Anti-CHO HCP Detection Kit
Time to result	210 min	62 min on Octet® RH96 system
		75 min on Octet® RH16 system with Octet® AS instrument
		90 min on Octet® R8 system with Octet® AS instrument
Dynamic range	1–100 ng/mL	0.5–200 ng/mL
Precision (CV)	15–25%	5–10%

Table 1: Comparison of overall assay performance for HCP analysis on Octet® systems and ELISA for 96 samples.

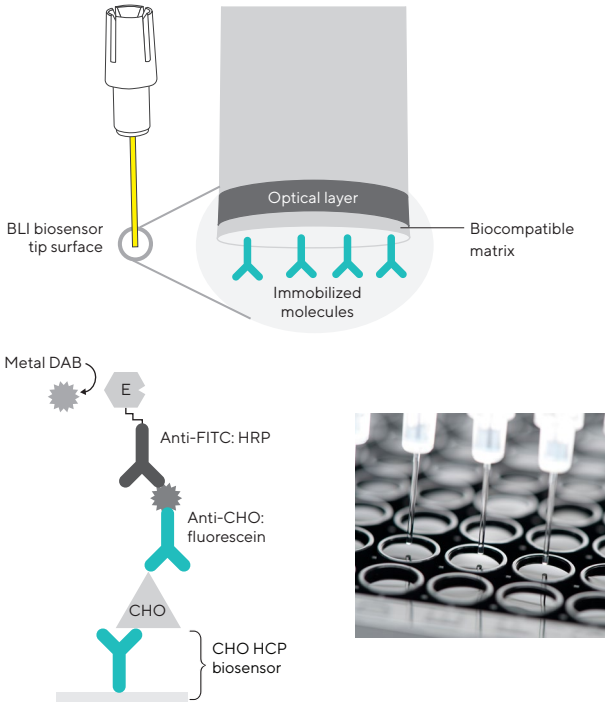

Figure 2: Host cell protein (HCP) detection lay-out on the Octet® platform. The biosensors come pre-immobilized with anti-CHO antibody.

Residual Protein A (RPA) Detection

A common challenge in bioprocessing is the copurification of antibody-based biologics with Protein A leaching off purification columns. Similar to HCPs, these proteins can affect the efficacy of the drug molecule and need to be detected and cleared. Sartorius' residual Protein A detection kit has a highly simplified workflow compared to traditional methods. The commonly used heat denaturation and sample centrifugation steps which can result into high process variability have been removed resulting into a significantly reduced assay time (Figure 3). This combined with the throughput and the automation capabilities of the Octet® instruments especially the Octet® RH96 and the RH16 systems, results into a rapid assay; 96-samples can be analyzed in under 2 hours on the Octet® RH96 system. The Sartorius kit can be adopted for the detection of leached Protein A from a resin that utilizes either recombinant Protein A or MabSelect Sure.

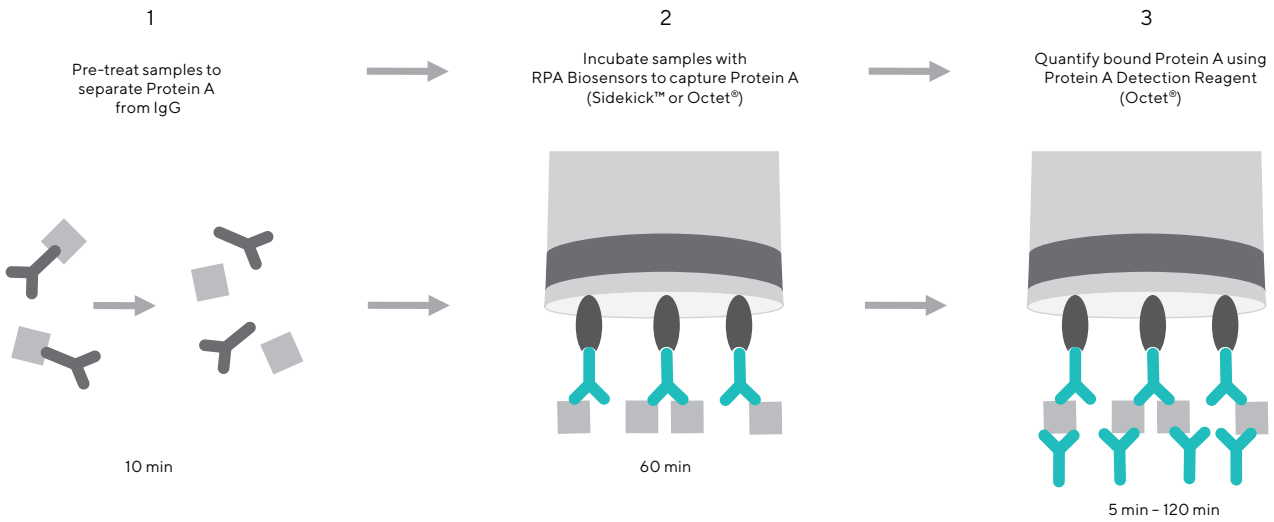

Figure 3: Residual Protein A workflow on the Octet® platform, no heating step is required. 1) Samples are treated with X and no heating step is required. 2) Separated samples are then incubated with RPA biosensors for 60 mins to capture free protein A. 3) A secondary Ab is then used to quantify the concentration of residual protein A from the sample.

References

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2. High-Throughput Sialylation Measurement Using Lectins on an Octet® Platform for Clone Screening, Jonnalagadda KN, et al., Analytical Methods, 8(39):7193-8, 2016.

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