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Enhancing Efficiency and Economics In Process Development and Manufacturing of Biotherapeutics

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Abstract

The high costs of discovery, development and production of therapeutic drugs necessitate the need for improved process efficiencies and economics. Analytical tools that circumvent traditional biologics analysis processes such as the need for labeling of reagents while allowing for real time visualization of data can help save development time and facilitate improved efficiencies during process development. Octet® instruments are used in a wide range of applications in process development and biotherapeutics manufacturing; including in early bioprocess development applications such as cell line development and clone selection and in downstream applications such as the determination of dynamic binding capacity for affinity purification columns. The technology's ability to monitor binding interactions in real-time coupled with its capability to analyze samples in their crude matrix and in high throughput can help shorten development and analysis times dramatically leading to significant cost savings. In this application note, insights from Octet® instrument adopters at GlaxoSmithKline and Aragen Biosciences illustrate multiple examples where the Octet® system has been used to improve process efficiencies and highlights its advantages over HPLC and ELISA in various segments of process development.

Introduction

Analytical techniques that measure protein quantity and quality are used in nearly all stages of research, process development and manufacturing of biotherapeutics. UV spectroscopy, ELISA and HPLC have been in use for decades for protein quantitation in physiological and process samples, and continue to be the workhorses despite their many limitations. To characterize the functional activity of proteins during biotherapeutic development, label-free biosensor-based binding assays are increasingly being utilized. The high cost and lengthy times associated with drug discovery and development have forced biopharm companies to improve the efficiency and economics in all stages of development. These key drivers have fueled the search for innovative analytical techniques that provide improved performance and speed without increasing costs. Biopharmaceutical companies have enthusiastically adopted Sartorius' Octet® systems due to their broad utility in protein quantitation and functional characterization combined with enhanced throughput, decreased sample preparation requirements, and low cost of operation. This white paper describes the use of Octet® instruments for protein quantitation, particularly in the areas of process development and quality control.

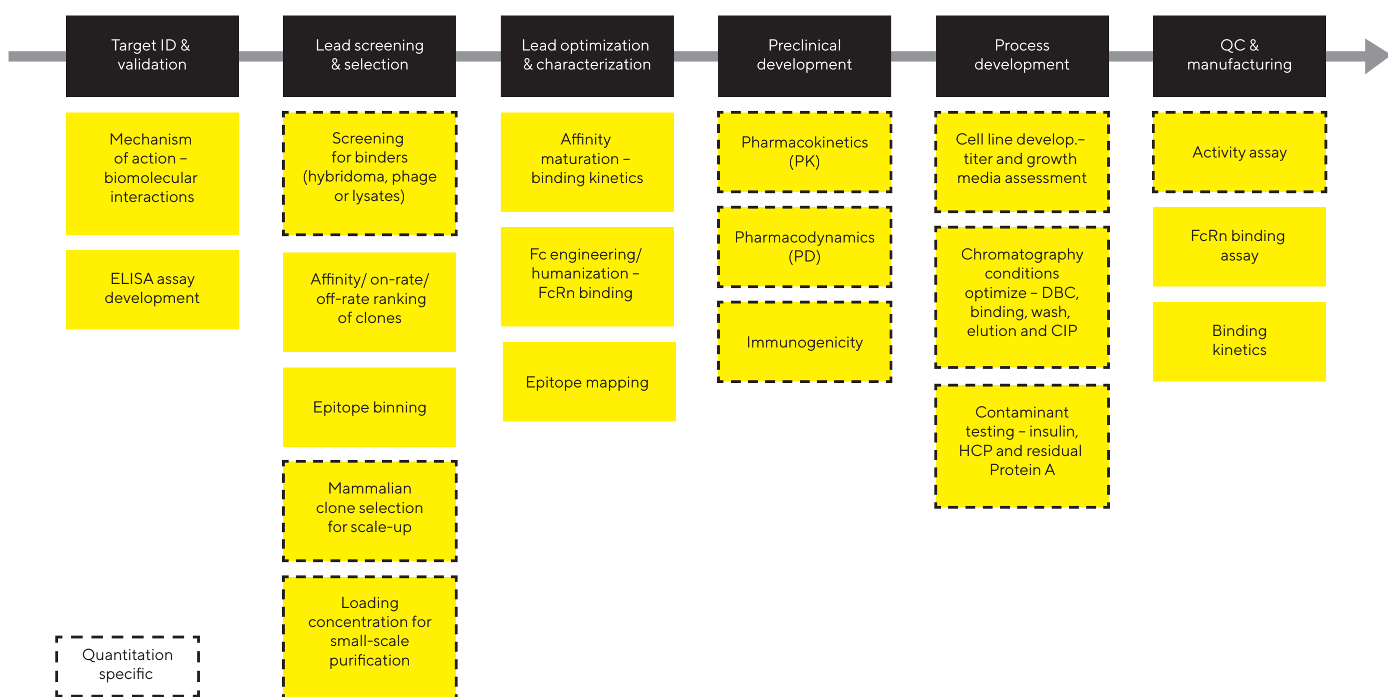


Figure 1: Applications of Octet® instruments in the drug research and development process.

Advantages over ELISA and HPLC

The principles of concentration measurement with an Octet® system are similar to established immunoassays such as ELISA and HPLC. However, protein quantitation protocols on the Octet® platform provide several advantages. The Octet® platform monitors binding of proteins from solution to a biosensor surface in real time, without need for labels or other detection reagents. This real-time monitoring of binding interactions enables clear discrimination between specific and non-specific binding signals, which can shorten assay development times dramatically. Octet® assays are also much faster: quantitation of a 96-well plate requires 15–30 minutes, or 60 minutes for a 384-well plate, depending on the instrument model. Figure 2 provides a comparison of analysis times. Analysis of 70 samples on an Octet® R8 system requires as little as 55 minutes including operator hands-on time, whereas ELISA or HPLC assays require at least 22 hours including several hours of analyst involvement. Samples run on Octet® systems are also recoverable, so that researchers may save and reuse precious sample for other experiments. In addition, Octet® assays are not affected by absorption interferences in colored samples or by light scattering with turbid samples, enabling measurement of analyte concentration in crude matrices such as cell culture supernatant, cell lysate and serum. Octet® concentration assays are complemented by the platform's ability to measure functional activity. For example, titer for a monoclonal antibody (mAb) can be determined using biosensors coated with Protein A, while the functional activity of the mAb can be assessed in a second assay step involving binding to its specific antigen. In contrast, HPLC and A280 spectroscopy can determine only the total protein concentration of a sample, and separate assays must be used to measure biological activity.

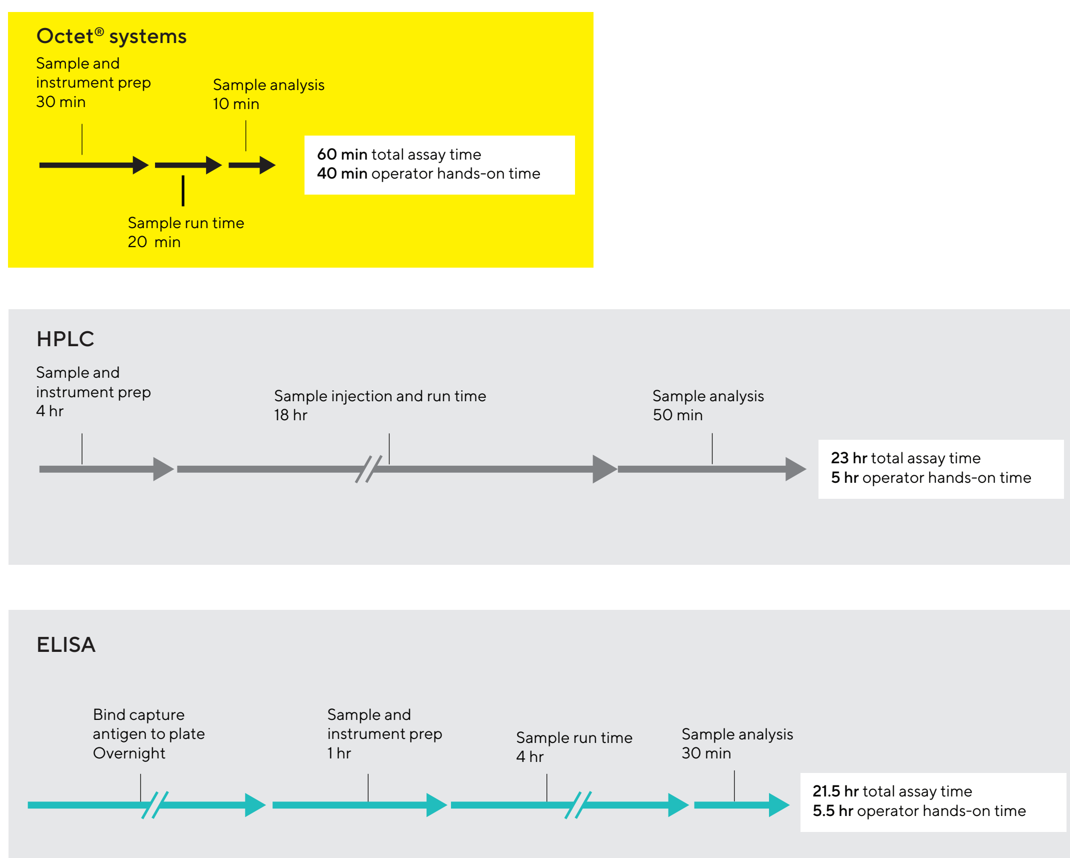


Figure 2: Comparison of protein quantitation in complex matrices using Octet® systems and alternative methods.

Bio-Layer Interferometry Technology (BLI)

BLI technology monitors and analyzes the interference pattern generated from the reflection of white light from two different surfaces: a layer of immobilized protein on the biosensor tip and an internal reference layer (Figure 3). Any increase or decrease in the number of binding molecules to the biosensor surface produces a change in optical thickness that causes a shift in the interference pattern. Unbound molecules in complex matrices and changes in the refractive index of the surrounding medium have minimal effect on the interference pattern. BLI technology simplifies protein quantitation by enabling specific measurement in complex samples. The one-step Dip and Read assay format uses native proteins, without need for labels or secondary reagents.

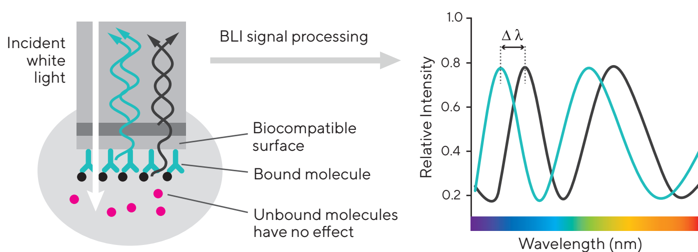
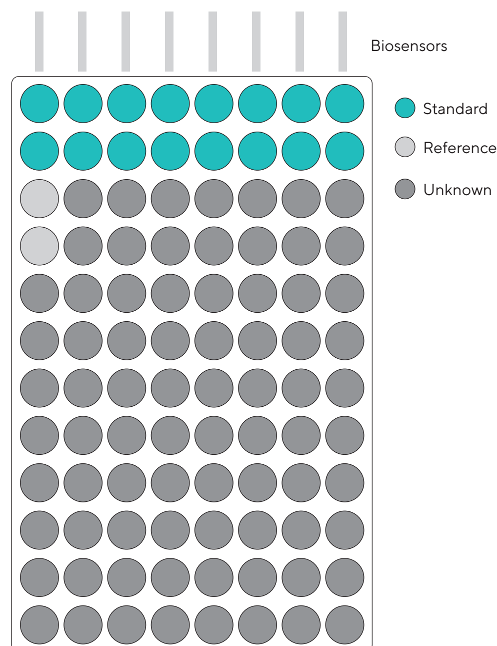
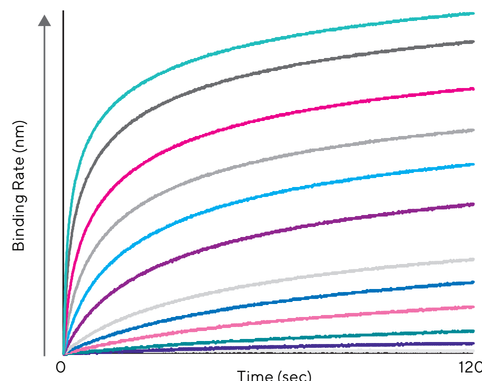


Figure 3: Bio-Layer Interferometry is an optical analytical technique that analyzes the interference pattern of white light reflected from two surfaces. Changes in the number of molecules bound to the biosensor causes a shift in the interference pattern that is measured in real time.

Concentration Measurement

Accurate determination of biologically relevant protein concentrations is essential to several areas in the biopharmaceutical industry including research, bioprocessing, quality control and manufacturing. The Octet® platform uses a simple Dip and Read approach for rapid analysis of samples in 96 and 384-well microplate formats. The concentration of the target protein or antibody in a sample is determined via a direct binding or sandwich assay. Biosensors coated with a capture molecule, called the ligand, are dipped into solutions containing the analyte in a highly parallel, automated method to measure binding interactions. In a typical quantitation assay, a standard curve is generated using known amounts of the protein analyte, and unknown sample concentrations are interpolated from the standard curve (Figure 4). Concentration can be calculated from the initial binding rate of the interaction which is based on the initial slope or from the binding rate at equilibrium.





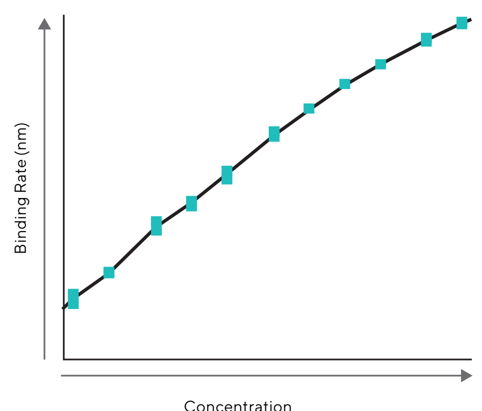


Figure 4: A typical quantitation assay setup. Biosensors dip into standards run in duplicate to obtain binding rate (nm) vs. time (sec) curves. The initial slope of the interaction is used to create the standard curve of the binding rate vs. concentration. The concentration of an unknown sample is then interpolated from the standard curve.

Quantitation Applications for Drug Development

Research and Early Bioprocess Development

The Octet® platform is a useful tool for cost-effective protein expression screening in research and early bioprocess development with several significant benefits.

Octet® platform advantages

- Antibody and protein concentrations can be determined in crude matrices, such as cell lysates or hybridoma supernatants, saving time and resources when processing a large number of samples.
- Octet® assays have a dynamic range of greater than two orders of magnitude, enabling a single quantitation assay to be utilized across all development stages – from early cell culture to production bioreactors.
- Octet® systems perform rapid quantitation with minimal user involvement. 96 samples are analyzed in as little as 20 minutes, and 384 samples in 70 minutes. With additional plate handling automation, Octet® RH16 systems can process more than 1200 samples per day.
- Samples are analyzed in a non-destructive method and are fully recoverable, which is advantageous when working with low sample volumes and precious samples.
- Octet® systems are easy to learn and operate. Multiple analysts can operate the instrument with minimal training, allowing rapid integration of these systems into laboratory workflows.

Early clone selection

In clone selection, thousands of hybridoma or phage clones are screened to determine positive binding clones and their protein secretion levels. Titer measurements are used to select high-producing clones and to normalize the functional activity of these clones in crude matrices. Integration of an Octet® system into the antibody discovery workflow affords increased screening throughput. With Octet® RH16 and Octet® RH96 instruments, automated plate handling can also be added to achieve even higher throughput. Octet® quantitation assays are also used to determine loading levels of chromatography columns for small-scale purification.

Cell line development

Harvest samples are screened on Octet® systems to select high-expressing clones during various scale-up procedures involving 96-, 48-, 24- and 6-well plates, fed batch shake flasks, and bioreactors (Figure 5). Octet® assays also are used to determine protein levels during media development for fed-batch and bioreactor processes (Figure 5). This is performed by comparing protein secretion levels fol-

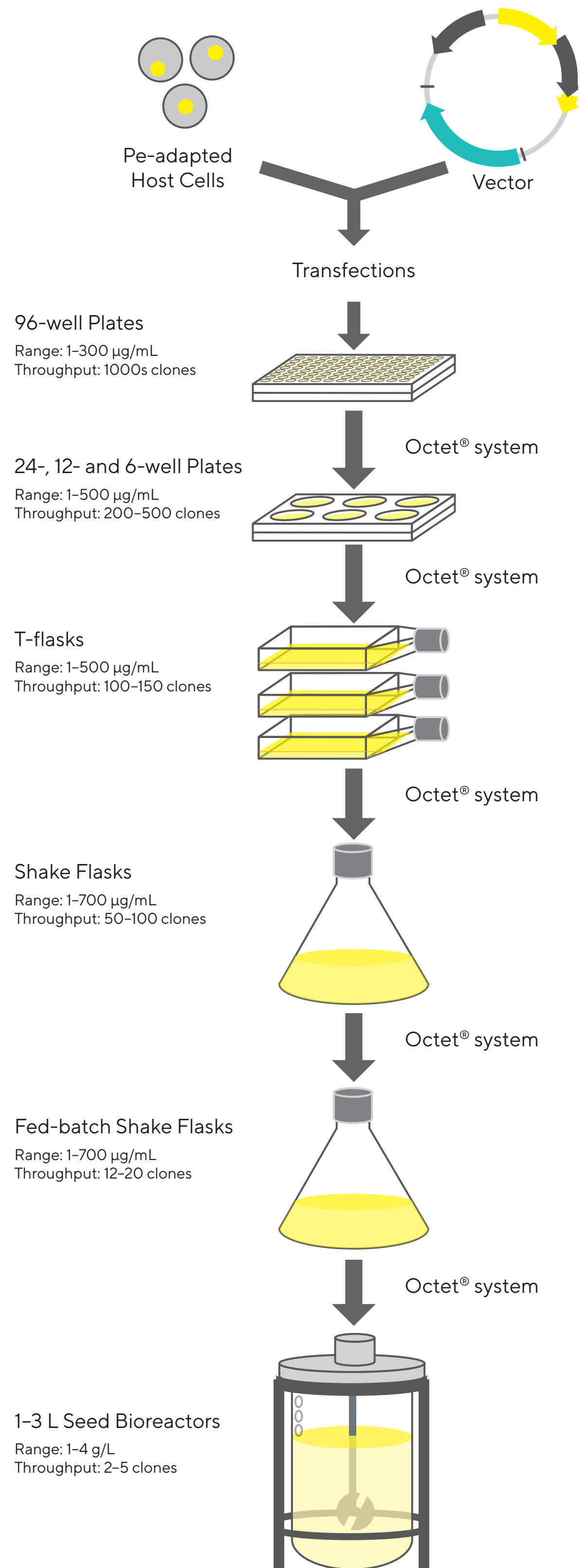


Figure 5: Protein titer assessment and growth media optimization using the Octet® system at different stages of cell line development.

lowing variations in feeding regimes, strategies and concentrations. Data acquisition and subsequent data analysis can be performed rapidly for hundreds of samples, bypassing traditional processing bottlenecks. Please see Sartorius Application Note "Fc-Fusion Protein Quantitation in Cell Culture Supernatants" for more information.

Downstream Process Development

Efficient development of manufacturing processes for antibodies and recombinant proteins is a critical need for biopharmaceutical companies. Increasingly stringent regulatory requirements targeting better understanding and control of manufacturing processes are expected to impact product quality and performance. The Octet® platform can quickly determine the impact of multiple process variables at different stages of the purification process, and help identify optimal conditions that provide protein product with the desired yield, binding specificity and potency (Figure 6). Pre-configured reagents and protocols are available for rapid quantitation of protein products, host cell proteins (HCP), and residual Protein A levels during purification processes.

Octet® platform advantages

- One Octet® instrument can be used to measure protein titer, host cell proteins and residual Protein A contaminant levels.
- Octet® assays are faster to develop and run than ELISA and HPLC assays.
- Octet® assays can be automated with robotic and liquid handling systems for complete, walk-away screening.

Dynamic binding capacity (DBC) of chromatography columns

Affinity chromatography often is the first major purification procedure performed on harvested cell culture samples in downstream bioprocessing. The dynamic binding capacity (DBC) of an affinity chromatography column is defined as the amount of protein that will bind to the column resin under a defined condition. DBC is determined by continuously loading a sample containing a known concentration of target protein and monitoring this protein in the flow-through fractions. Quick determination of DBC using HPLC or A280 spectroscopy is hampered by the presence of large amounts of host cell proteins in the flow-through fractions. Specific detection of the protein of interest among contaminants is straightforward with Octet® systems, reducing the time required to optimize purification conditions (Figure 6).

Binding, wash and elution conditions

Numerous chromatography binding and elution conditions are tested during optimization studies, including different buffer compositions, salt, pH, operating temperature and sample injection volume. High-throughput tools, such as mini columns and 96-well filter plates, often are used to screen these process variables. The impact of different conditions on product titer and quality can be analyzed rapidly and effectively on Octet® systems, speeding identification of optimal chromatography conditions (Figure 6).

Contaminant testing

Downstream purification processes must remove host cell proteins, residual Protein A and residual DNA impurities. According to guidance from regulatory authorities, host cell

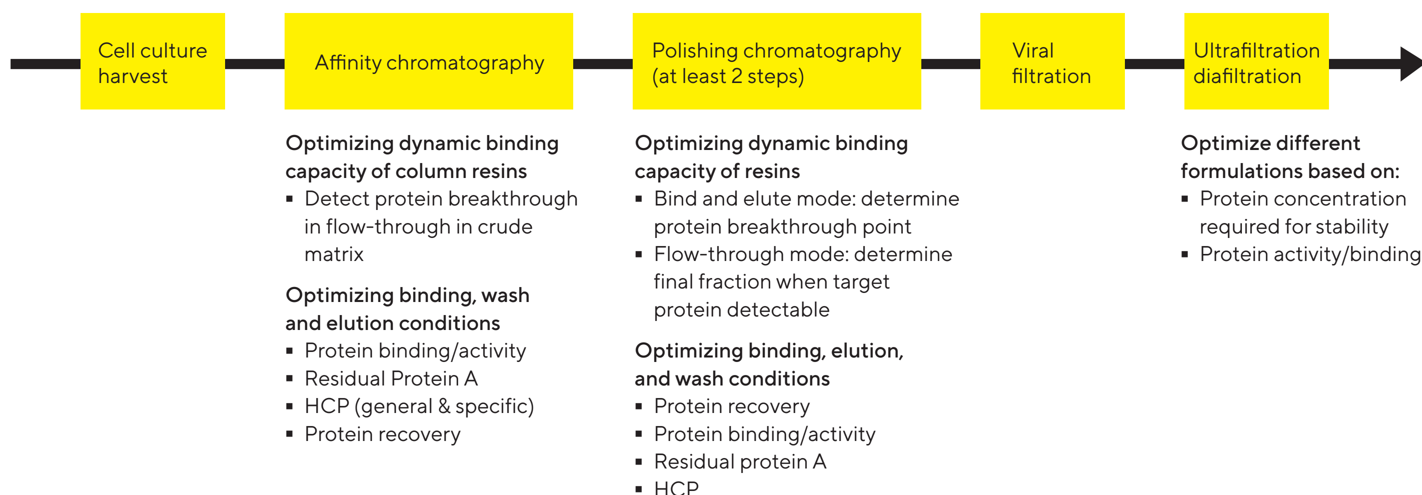


Figure 6: Use of Octet® systems in the downstream purification process of proteins and antibodies.

proteins in a drug substance should be below detectable levels using a highly sensitive analytical method, and as a rule this level should not exceed 100 ppm. The type of assay required for HCP determinations depends on the phase of clinical studies for which the material is produced. For earlier clinical phases, a generic assay may be sufficient. However, a process-specific HCP assay generally is required for phase 3 and later studies. Leached Protein A is another contaminant of concern in process development. The elution of antibodies during Protein A chromatography requires acidic conditions, which in turn can accelerate leaching of Protein A from the column. Residual Protein A levels should not exceed 10 ppm in the final drug product.

Customer Highlight: GlaxoSmithKline

The analytical lab at GlaxoSmithKline incorporated a generic HCP assay on the Octet® QK384 system to streamline their workflow in process development. The automated Octet® HCP assay required minimal analyst intervention and provided more accurate and precise results than their manual ELISA assay (Table 1). Hands-on time for preparation and processing of 1–3 assay plates was reduced to 30 minutes from the previous 2.5 hours with manual ELISA, and antibody consumption decreased by 40%.

More information on the development of the HCP assay on Octet® systems can be found in Technical Note, Host Cell Protein Dectection on the Sartorius website.

Process development assays for residual Protein A and product titer can be fully automated on Octet® RH16 systems using external liquid handling platforms. The Octet® assay for leached Protein A is highly sensitive with a LLOQ of 0.20 ppm, has >2.5 logs of dynamic range, and is faster than competing methods. A residual Protein A assay on the Octet® RH16 system can be completed in 1 hour and 45 minutes per plate with minimal analyst involvement, compared to a minimum of 3.5 hours for ELISA (including significant analyst hands-on time). For more information on the Octet® residual Protein A quantitation protocol, see Technical Note, Dip and Read Residual Protein A Detection Kit on the Sartorius website.

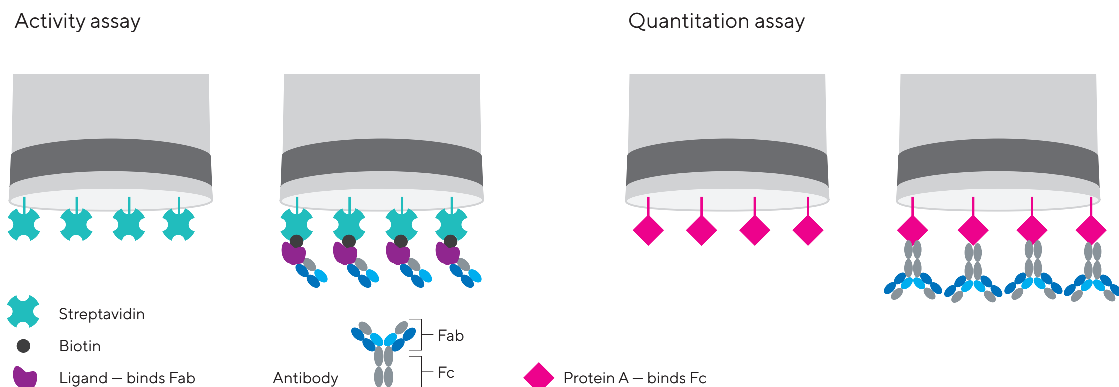


Figure 7: An activity assay can be developed on the Octet® platform by immobilizing a specific biotinylated ligand on the biosensor and then detecting binding of an analyte, Fab or protein. In the quantitation assay, mAb titer is determined using Protein A-loaded biosensors, which does not measure mAb activity towards its target.

Quality Control

Octet® systems provide robust and highly reproducible assays for protein concentration and functional activity, and are suitable for operation in quality control and manufacturing environments. Protein activity and various kinetic assays are used to support in-process testing, drug potency, lot-to-lot variability and stability studies.

Octet® platform advantages

- Octet® systems are designed for GLP/GMP environments, and provide 21 CFR Part 11 compliance tools.
- Octet® assays provide detailed information about the binding behavior of protein products, and reveal subtle differences in binding activity between production lots.

Customer Highlight: Aragen Biosciences

Aragen Biosciences created a stable and scalable CHO cell line, purification platform and manufacturing process for a particular product in a GMP environment. They developed an Octet® assay to compare the activity and quality of a new product lot (Lot 2) with a reference lot (Lot 1) throughout their bioprocess and manufacturing processes. The assay involved loading a biotinylated ligand on Streptavidin Biosensors, and measuring binding interaction of the ligand with the protein analyte. As seen in Figure 8, Lot 2 contained a large second peak that was absent in the Lot 1 reference material. The second peak in Lot 2 exhibited a slower on-rate and much faster off-rate, indicative of a less-active fraction (Figure 9). Octet® system activity data results were confirmed with a cell-based assay, and Aragen was able to modify their production conditions to significantly reduce this second peak fraction.

- Octet® quantitation assays provide a direct measure of the biological activity of the analyte(s) (Figure 7).
- Octet® assays can be easily transferred to manufacturing operations.

Activity assays

An activity assay is generally utilized during process development, QC and manufacturing to compare various prepared lots of the drug molecule, as well as its stability. Activity assays are critical because they differentiate active protein from inactive or clipped variants, as those species will not bind the ligand. Active protein concentration can be determined using a binding assay on the Octet® platform by immobilizing a specific ligand against the target analyte onto the biosensor, and then measuring its binding interaction with the analyte as shown in Figure 7.

Benefits of automated Octet® CHO HCP assay compared to manual ELISA

Benefit	Details
Precision	Liquid handling robot reduces pipetting variation inherent in manual pipetting
Reliability	Method performed exactly the same each time
Streamlined process	Worklist drives robotic method and creates sample plate importation files Robotic method automatically creates and executes Octet® method file
Walk away	No analyst intervention needed to complete method after instrument loaded and diluent volumes are checked
Washing steps	No washing steps needed and plate washer integration not required
Analysts involvement	Automated Octet® → ~30 minutes for 1–3 assay plates Manual ELISA → ~2.5 hours per assay plate
Throughput	3 assay plates can be run in ~5 hours 38 samples/plate in duplicate wells > 108 samples in 3 plates
Antibody consumed	Re-use of coating antibody can significantly reduce consumption over multiple assay plates

Table 1: Benefits of automated Octet® CHO HCP assay compared to manual ELISA summarized by GSK.

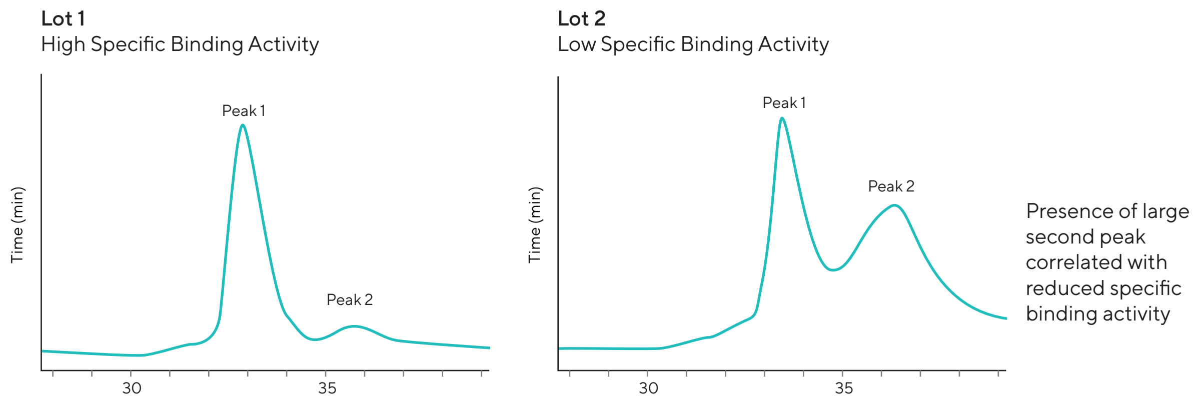


Figure 8: HPLC spectra of Lot 1 and Lot 2 of a drug molecule. Lot 2 was made by Aragen Biosciences and had an additional peak (Peak 2) compared to the reference lot (Lot 1) provided by their customer. Data provided courtesy of Aragen Biosciences.

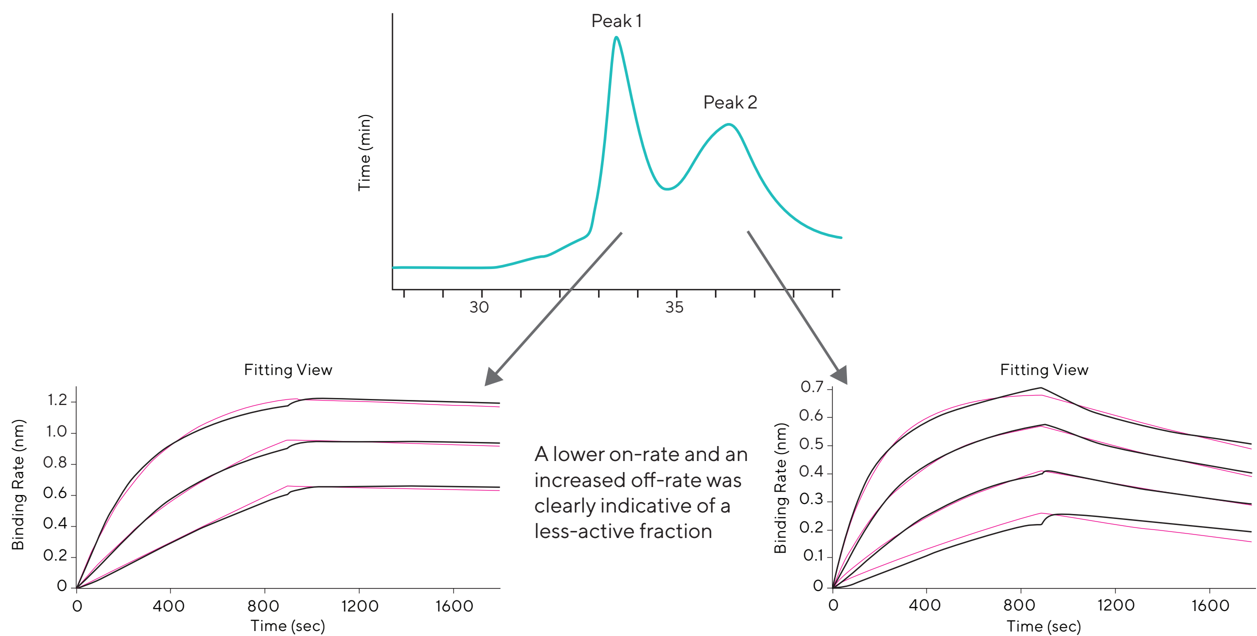


Figure 9: The Octet® binding kinetics or functional assay demonstrated that Peak 1 was the active fraction. Peak 2 was the less-active fraction, with a lower on-rate and a much faster off-rate in a binding experiment. Data provided courtesy of Aragen Biosciences.

Conclusion

Octet® systems deliver comprehensive characterization of biotherapeutics, as well as rapid and reproducible determination of protein concentrations during different stages of the development process. Titer and functional activity assays on Octet® systems are useful for a broad array of applications in target identification, lead selection, process development, formulation development, quality control, and manufacturing. In early stages of drug development, Octet® systems provide the high throughput needed to screen through large libraries of candidate drug molecules. In later stages of process development and manufacturing, Octet® systems provide the required reliability, robustness and measurement accuracy. The broad utility of this single platform makes the Octet® instrument unique in its ability to deliver high value across a wide range of application needs in biopharmaceutical discovery, development and manufacturing processes.

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