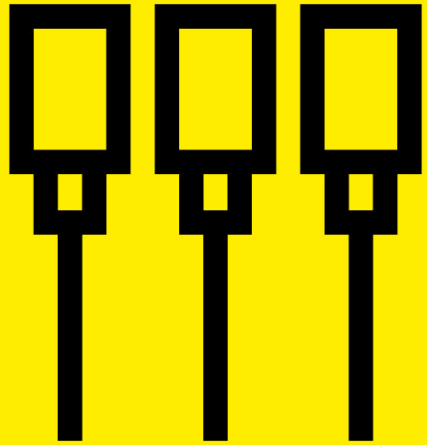
CQA



Simplifying Progress

Rapid and Efficient Measurement of Critical Quality Attributes and Parameters Using Octet® Bio-Layer Interferometry (BLI) Systems

SARTORIUS

Introduction

Critical quality attributes (CQAs) are defined as a physical, chemical, biological, or microbiological property or characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality.[1] These essential measurements include product-specific attributes such as size, charge, and glycosylation patterns as well as process-related impurities including host cell proteins (HCPs) and residual Protein A from chromatographic purification.

CQAs must be identified early in the discovery process, reflect the target product profile of the drug candidate, and continue to be refined through preclinical and clinical development phases. Traditional Analytical techniques used to measure CQAs include UV spectroscopy, enzyme-linked immunosorbent assays (ELISAs), and high-performance liquid chromatography (HPLC).

The Octet® Bio-Layer Interferometry (BLI) platform offers an excellent alternative to assays performed using these traditional time- and labor-intensive methods. Label-free assays are fast, fully automated, require only limited user intervention, provide a simplified workflow, and are used throughout biotherapeutic discovery and development to simplify and streamline measurement of CQAs.[2,3]

This compendium summarizes the use this fluidic-free instrument apporach for a variety of CQA applications across the drug discovery and development workflow.

Application Note: Converting an ELISA Assay

The Principle of Bio-Layer Interferometry (BLI)

BLI is an optical technology that measures changes in interference patterns between light waves. Octet® BLI systems measure light interference originating from the tip of a biosensor surface where light wavelengths reflect from two layers, a biocompatible layer at the end of the biosensor and an internal reference layer (Figure 1). Incident white light reflecting from the two layers interfere constructively or destructively depending on the thickness of the molecular biolayer at the biosensor tip. The spectral pattern of the reflected light changes as a function of the optical thickness of the molecular layer, such as the number of molecules bound to the biosensor surface. This spectral shift is monitored at the detector and reported on a sensorgram as a change in wavelength (nm shift). Monitoring the interference pattern, or spectral shift, provides real-time kinetics data on molecular interactions.

Dip-and-read biosensor tips used in Octet® BLI systems are coated with a biocompatible matrix that minimizes non-specific binding while providing a uniform and non-denaturing surface for biomolecules. The biosensor with immobilized capture ligand moves over the 96- or 384-well plate and is dipped into the sample; elimination of complex fluidic pathways allows characterization of interactions directly in complex matrices and non-purified samples.

The advanced technology of Octet® BLI systems enables the user to:

- Acquire real-time binding kinetics data to measure the rate of association (k_a), the rate of dissociation (k_d) and affinity constants (K_D)
- Measure up to 96 samples simultaneously with fully automated assays
- Rapidly identify optimal assay conditions using up to 96 channels for multiple conditions and reaction configurations in a single run
- Generate data from quantitation assays in real time
- Detect binding of a wide range of analytes, from small molecules to nanoparticles
- Recover precious or low-availability samples as binding reagents are not added directly to the sample and materials are minimally consumed.

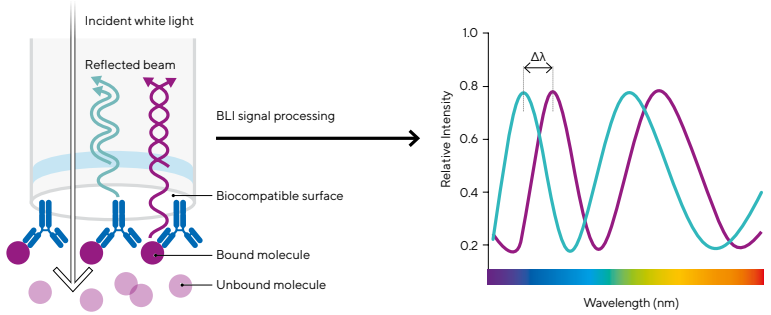


Figure 1: Octet® systems with BLI technology measure the difference in the wavelength of reflected light (Δλ) between the two surfaces on the biosensor.

Click to Expand

The Principle of Bio-Layer Interferometry (BLI)

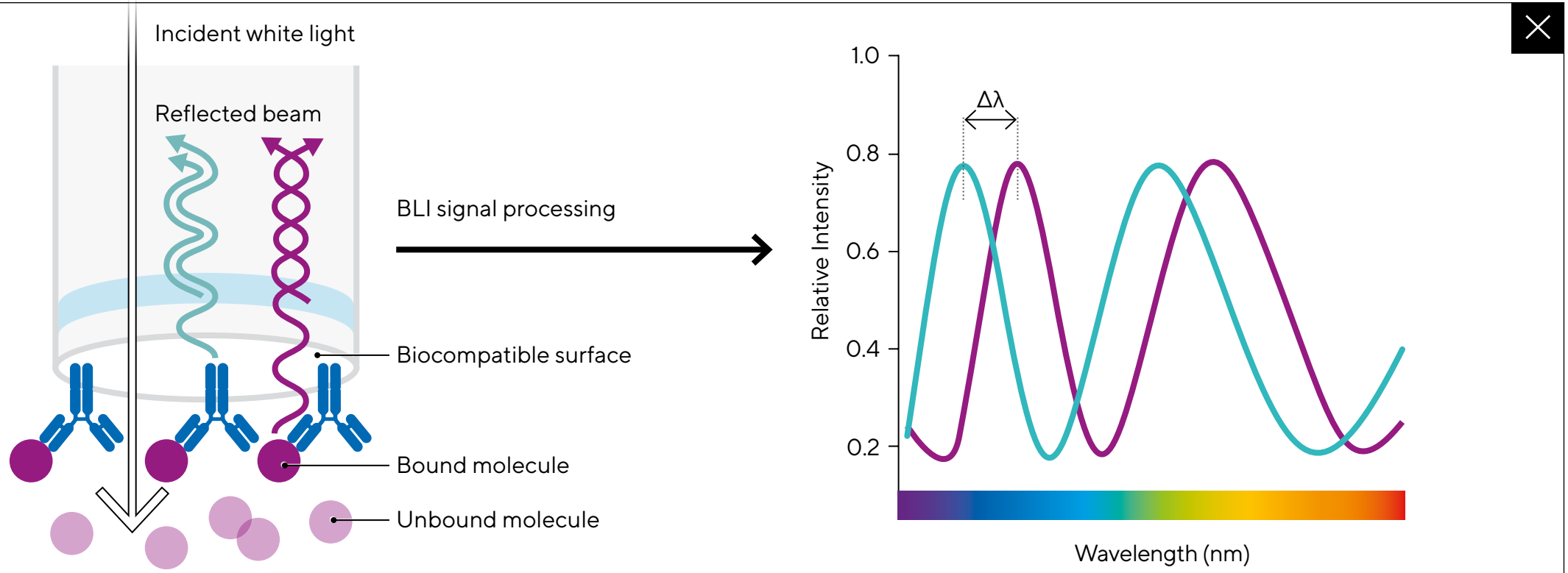


Figure 1: Octet® systems with BLI technology measure the difference in the wavelength of reflected light (Δλ) between the two surfaces on the biosensor.

Elucidating Mechanisms of Action

Understanding the specific biochemical interaction through which a drug substance exerts its pharmacological effect is an important part of drug development. Kinetic rate constants and equilibrium affinity are two key parameters for understanding the mechanism of action (MOA) of a potential drug against its target and guiding selection of candidates for further development. In addition to identifying and advancing lead molecules, kinetics characterization is also critical in comparing biosimilar molecules to their originator counterparts. For biosimilars, evaluation of product quality attributes, especially functional equivalence assessed through ligand binding studies, is of primary importance in ensuring similar clinical safety and efficacy in patients.

With outstanding flexibility and a broad range of assay formats, the Octet® BLI platform is well-suited to elucidate the MOA of different therapeutic modalities.

Additional Information (click to expand)

- Affinity and Avidity of Anti-Viral Antibodies +
- Interactions of Therapeutic Antibodies with Fcγ and Neonatal Fc Receptors +

Elucidating Mechanisms of Action

Affinity and Avidity of Anti-Viral Antibodies

As part of a study to identify antibodies targeted against MERS-CoV and their role in virus adaptive evolution, Tang, et al, used the Octet® BLI platform to measure binding rate constants (equilibrium dissociation constant (K_D, k_a, and k_d) and neutralization activity of anti-spike single chain variable fragment – Fc fusion proteins (scFv-Fcs) and IgGs.[4] Anti-MERS-CoV spike neutralizing antibodies from a nonimmune human Ab-phage library were isolated; the antibodies bound to different epitopes with subnanomolar to nanomolar affinity and neutralized MERS-CoV infection.

While antibody affinity maturation is a critical step in development of functional antiviral immunity, accurate measurement of affinity maturation of polyclonal serum antibody responses to particulate antigens such as virions is challenging. Tsuji, et al, used the Octet® platform to assess the avidity of dengue virus-specific antibodies elicited in response to a tetravalent dengue vaccine.[5] After validation of the assay using anti-dengue monoclonal antibodies, the assay was used to assess avidity of antibody responses to a tetravalent dengue vaccine candidate in phase 2 clinical trials conducted in dengue-endemic regions.

Elucidating Mechanisms of Action

Interactions of Therapeutic Antibodies with Fcγ and Neonatal Fc Receptors

A major mechanism of action for therapeutic monoclonal antibodies is the initiation of effector function via binding to Fc gamma receptors (FcγRs) expressed on the surface of immune effector cells. The ability of these antibodies to bind FcγRs can greatly impact their safety and efficacy, and as such, efforts to analyze and enhance Fc interactions with FcγRs are often the focus of development processes.

The Octet® BLI platform offers a high-throughput, rapid, flexible, and sensitive approach for the accurate and reliable measurement of the binding affinities of Fc gamma receptors to monoclonal antibodies. These interactions can be evaluated via full kinetic analysis, steady state analysis, or by measuring relative binding or potency. Kinetic assays can be extremely useful for comparing the Fc receptor binding activity of antibodies produced from different clones, different production processes, or prepared in different formulations. In addition, these methods can potentially be adapted to function in comparability studies and qualified or validated GMP lot release and stability studies.

Methods for producing high quality FcγR kinetic data using a variety of assay formats, along with recommendations for assay optimization, data acquisition, curve fitting, and data analysis have been detailed by Tobias, et al.[6] Considerations such as biosensor selection, assay orientation, choice of buffers, ligand immobilization and regeneration conditions and their potential impact on results are also described.

Geuijen, et al, also described measurement of interactions of therapeutic antibodies with fragment crystallizable γ (Fcγ) receptors and neonatal Fc receptors (FcRn) as indicators of antibody functional performance.[7] The goal of the study was to develop a screening assay that would rapidly measure IgG binding to different Fcγ receptors and FcRn as part of CQA assessments during lead optimization studies and in-process control, and for the biosimilarity assessment of IgGs during development and manufacturing.

Real-time data collection can also be used to measure affinity constants, a key property for determining the degree of interactions between candidate therapeutic molecules and their target receptors.[8] This information, together with the kinetics of binding, facilitates identification and selection of lead candidates during therapeutic drug development.

Cell Line Development and Titer

Cell line development requires screening of clones to find those that are stable, produce high yields of the biologic, and exhibit desired CQAs. The process typically includes the screening of thousands of clones to find the few that are stable, grow as expected, and produce high yields of the biologic. While expression level analyses such as titer screening are performed early in the process, other CQAs such as glycan characterization, are often assessed only later in the development process due to a lack of appropriate and high-throughput analytical techniques that can be used as a quick screen.

The Octet® BLI platform can be used to detect real-time binding of molecules both as a means of quantification and for kinetic analysis, enabling rapid selection of optimal clones, accelerating the cell line development process.[9] The versatility allows titer and glycan screening to be performed on the same samples; glycosylation is a CQA as it can impact pharmacokinetics properties and stability (Figure 2).

Additional Information (click to expand)

- Functional Evaluation of Bispecific Antibodies +
- Determination of Protein Concentration +

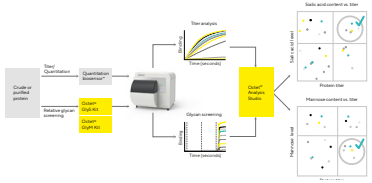
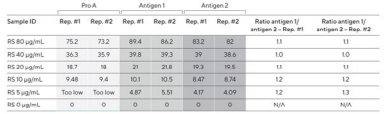


Figure 2: Workflow of simultaneous titer analysis and glycan screening.

+ **Click to Expand**



Sample ID	Pro A		Antigen 1		Antigen 2			
	Rep. #1	Rep. #2	Rep. #1	Rep. #2	Rep. #1	Rep. #2	Ratio antigen 1/ antigen 2 – Rep. #1	Ratio antigen 1/ antigen 2 – Rep. #2
RS 60 µg/mL	75.2	73.2	89.4	86.2	83.2	82	1.1	1.1
RS 40 µg/mL	36.3	35.9	39.8	39.3	39	38.6	1.0	1.0
RS 20 µg/mL	18.7	18	21	21.8	19.3	19.5	1.1	1.1
RS 10 µg/mL	9.48	9.4	10.1	10.5	8.47	8.74	1.2	1.2
RS 5 µg/mL	Too low	Too low	4.87	5.51	4.17	4.09	1.2	1.3
RS 0 µg/mL	0	0	0	0	0	0	N/A	N/A

Figure 3: Results of binding assay using bsAb.

+ **Click to Expand**

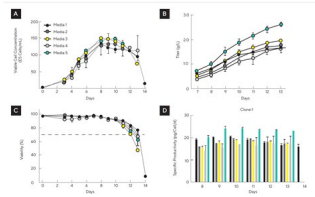


Figure 4: Time-course cell count and titer analysis for a clone in different media types.

+ **Click to Expand**

Cell Line Development and Titer

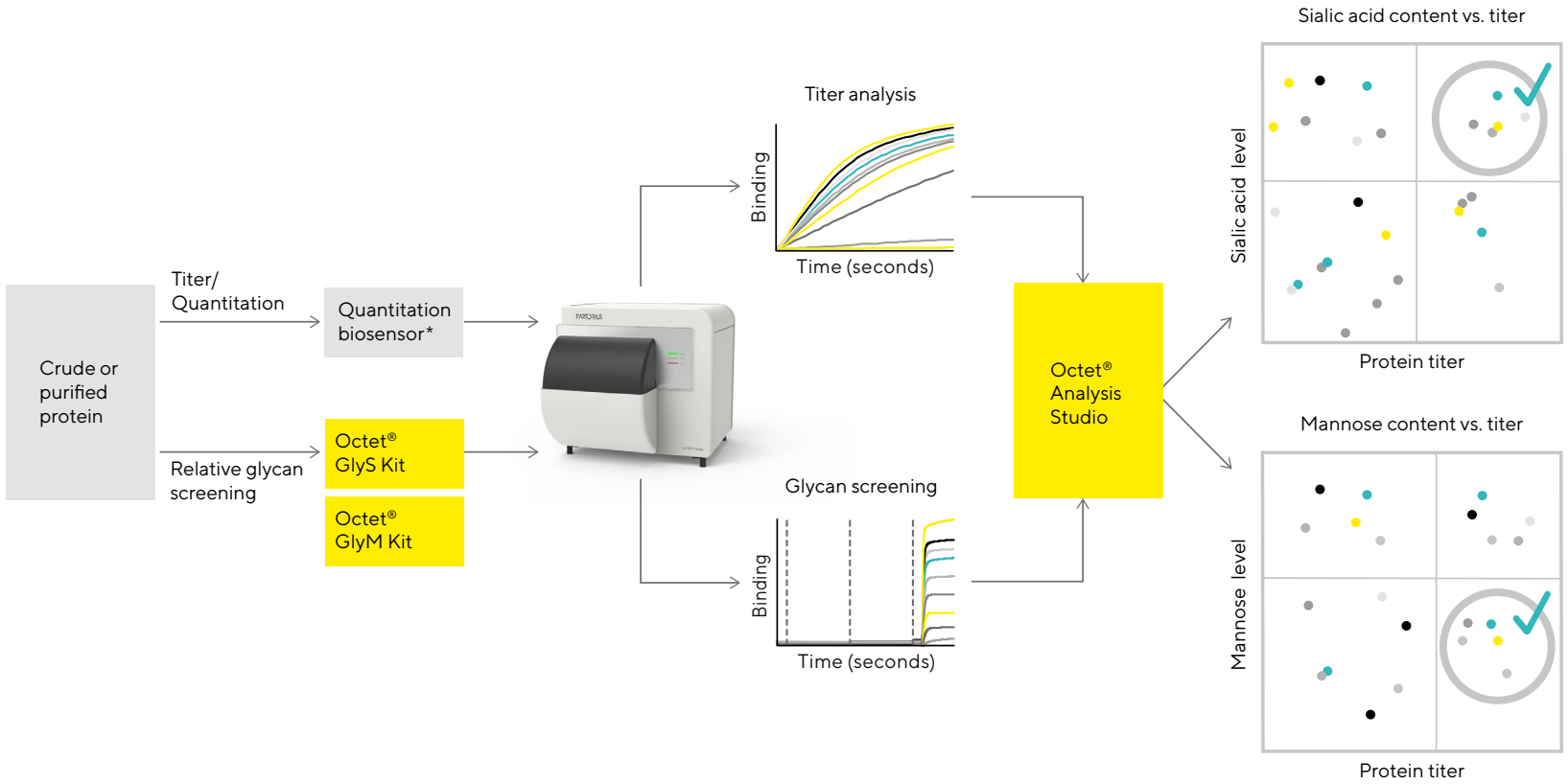


Figure 2: Workflow of simultaneous titer analysis and glycan screening.

Cell Line Development and Titer

	Pro A		Antigen 1		Antigen 2			
Sample ID	**Rep. #1**	**Rep. #2**	**Rep. #1**	**Rep. #2**	**Rep. #1**	**Rep. #2**	**Ratio antigen 1/ antigen 2 – Rep. #1**	**Ratio antigen 1/ antigen 2 – Rep. #2**
RS 80 µg/mL	75.2	73.2	89.4	86.2	83.2	82	1.1	1.1
RS 40 µg/mL	36.3	35.9	39.8	39.3	39	38.6	1.0	1.0
RS 20 µg/mL	18.7	18	21	21.8	19.3	19.5	1.1	1.1
RS 10 µg/mL	9.48	9.4	10.1	10.5	8.47	8.74	1.2	1.2
RS 5 µg/mL	Too low	Too low	4.87	5.51	4.17	4.09	1.2	1.3
RS 0 µg/mL	0	0	0	0	0	0	N/A	N/A

Figure 3: Results of binding assay using bsAb reference material and His-tag antigens corresponding to both antigen-binding sites. Consistency is shown in replicates demonstrating the accuracy of the standard curve.

Cell Line Development and Titer

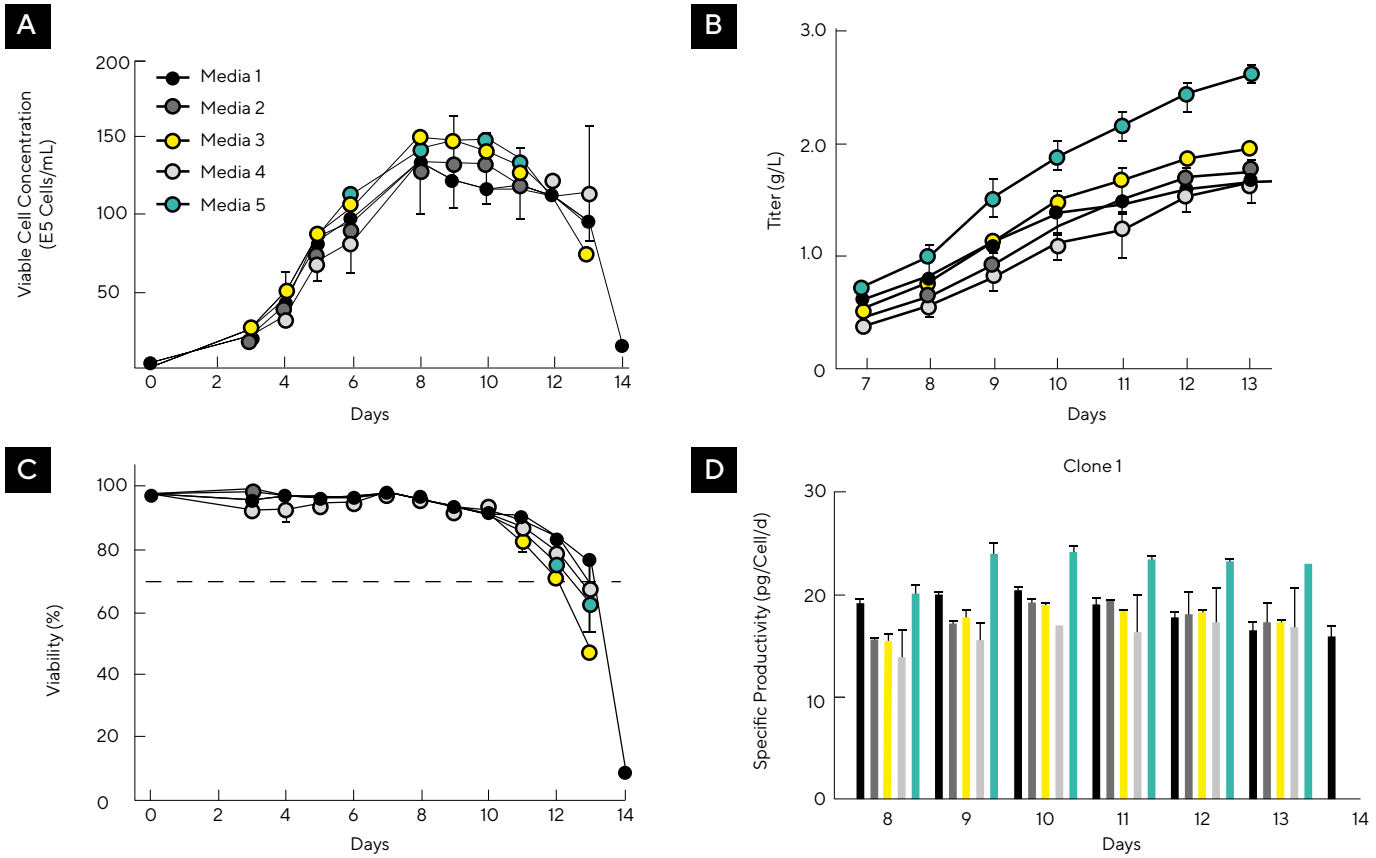


Figure 4: Time-Course cell count and titer analysis for clone 1 in different media types; cell count was performed using Vi-CELL XR while the Octet® BLI platform was used for titer determination.

Cell Line Development and Titer

Functional Evaluation of Bispecific Antibodies

Functional evaluation of bispecific antibodies (bsAbs) during expression clone screening presents a unique challenge as the producer cells can yield many different conformations including antibody fragments in symmetric or asymmetric arrangement. If screening is based only on Protein A titer, good pools and clone candidates might be discarded. A high-throughput assay can identify pools and clones with higher FcγR binding s of bispecific antibodies.[10] The assay consists of capturing bsAb by each antigen-binding site region sequentially using the correspondent antigen loaded onto the biosensor surface. The method offers an easy screening method and workflow that assesses bsAb interactions in a versatile, label-free, and easy-to-use format. High throughput can be achieved from the multiple simultaneous measures possible on the system and rapid assays times, such as binding optimization which can be determined in only 10 minutes.

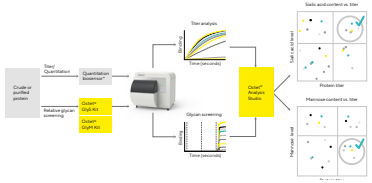
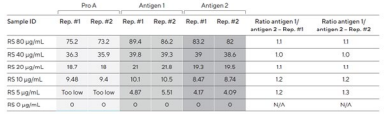


Figure 2: Workflow of simultaneous titer analysis and glycan screening.

Click to Expand



Sample ID	Pro A		Antigen 1		Antigen 2			
	Rep. #1	Rep. #2	Rep. #1	Rep. #2	Rep. #1	Rep. #2	Ratio antigen 1/ antigen 2 – Rep. #1	Ratio antigen 1/ antigen 2 – Rep. #2
RS 60 µg/mL	75.2	73.2	89.4	86.2	83.2	82	1.1	1.1
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RS 0 µg/mL	0	0	0	0	0	0	N/A	N/A

Figure 3: Results of binding assay using bsAb.

Click to Expand

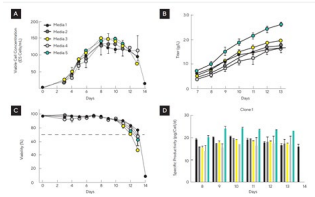

Figure 4: Time-course cell count and titer analysis for a clone in different media types.

Click to Expand

Cell Line Development and Titer

Determination of Protein Concentration Functional Evaluation of Bispecific Antibodies

The Octet® BLI platform can also be used for determination of protein concentration and titer in biological samples and offers important advantages over UV spectroscopy, ELISA and HPLC.[11] The platform monitors binding of proteins from solution to a biosensor surface in real time, without the 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 dramatically shorten assay development times; quantitation of a 384-well plate requires 60 minutes compared to more than twenty hours when using ELISA or HPLC. In addition, samples are recoverable, so they may be used again and the 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.

Implementation of the Octet® BLI platform alongside the Ambr® 15 cell culture system allows cell line developers to identify the best path forward for the choice of the top clone and the best media combination during the early stages of development.[12] Multiple media compositions and different clones can be rapidly assessed in single experiment (Figure 4).

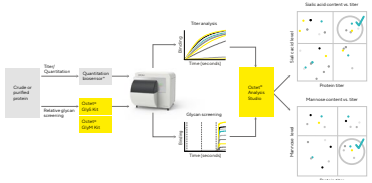

Figure 2: Workflow of simultaneous titer analysis and glycan screening.

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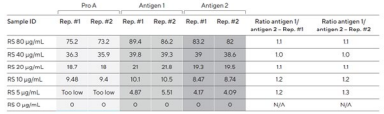

Sample ID	Pro A		Antigen 1		Antigen 2			
	Rep. #1	Rep. #2	Rep. #1	Rep. #2	Rep. #1	Rep. #2	Ratio antigen 1/ antigen 2 – Rep. #1	Ratio antigen 1/ antigen 2 – Rep. #2
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RS 0 µg/mL	0	0	0	0	0	0	N/A	N/A

Figure 3: Results of binding assay using bsAb.

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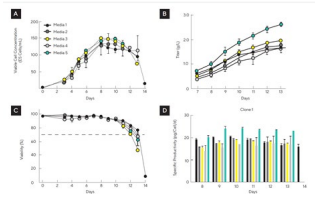

Figure 4: Time-course cell count and titer analysis for a clone in different media types.

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Post Translational Modification Assessment

Post-translational modifications of therapeutic proteins such as glycosylation have an impact on stability, solubility, and biological activity. Variability in glycosylation patterns, caused by differences in manufacturing processes, can adversely affect the biological activity and stability of proteins. As such, appropriate glycosylation is a CQA that must be demonstrated to ensure the safety and potency of commercial mAbs.

Current methods for the detection of glycans include staining procedures such as SDS-PAGE and affinity-based procedures such as specific lectin binding, antibody binding, or enzymatic cleavage methods. While staining procedures are fairly straight forward, they are however plagued with high propensity for non-specific binding making them less reliable as tools for specific glycan detection. An affinity-based approach is therefore more reliable as it utilizes molecules that bind specifically to a given glycan. Once detected, complete structural elucidation of glycoprotein oligosaccharides can be performed using a chemical, enzymatic, and chromatographic (HPLC) techniques combined with mass spectrometry (MS). While both MS and HPLC remain popular platforms for the characterization of glycosylation on proteins, they lack the throughput needed for early screening of CQA and are therefore not optimal for early clone selection where speed is desirable. Moreover, conventional techniques require sample purification, a process that can be prohibitively long. In contrast, the Octet® BLI platform is an easy to use, high-throughput approach for measuring glycosylation.[13,14] Unlike HPLC or MS techniques, the platform can also monitor other functional CQAs such as specific receptor binding from the same samples.

Additional Information (click to expand)

- Assay Method +
- Octet® GlyM Kit +
- Glycosylation CQA for Biosimilars +

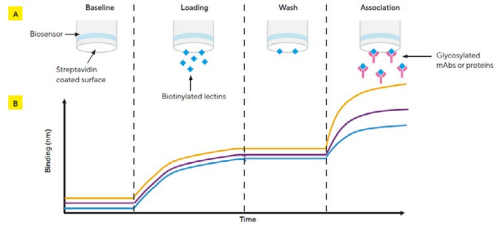

Figure 5: Binding which occurs at each assay step.

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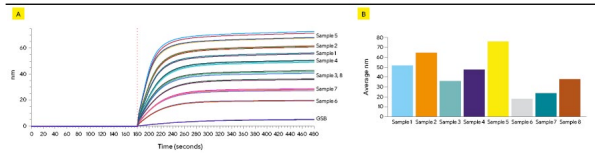

Figure 6: GlyM biosensors binding response.

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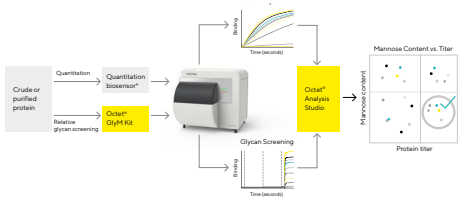

Figure 7: Schematic of mannose screening workflow.

+ **Click to Expand**

Post Translational Modification Assessment

Assay Method

Biosensors, coated with streptavidin, are first dipped into a solution containing biotinylated lectins. The strong affinity between streptavidin and biotin leads to the lectin molecules being immobilized to the surface of the biosensor. The custom biosensor is subsequently dipped into a sample containing glycosylated proteins. Only proteins with the complementary glycan structures will bind to the lectin-coated biosensors (Figure 5A). The interference patterns of white light reflected from the end of the biosensor and an internal reference layer are monitored. Figure 5B shows how the binding changes with each assay step. Ultimately, this allows the quantification of glycosylated proteins when a standard curve is used.

The effector functions of therapeutic antibodies are strongly affected by the specific glycans added to the antibody Fc domain during post-translational processing. Antibodies bearing high levels of N-linked mannose-5 glycan (Man5) have been reported to exhibit enhanced ADCC compared with antibodies with fucosylated complex or hybrid glycans.

Additional Information (click to expand)

- Assay Method +
- Octet® GlyM Kit +
- Glycosylation CQA for Biosimilars +

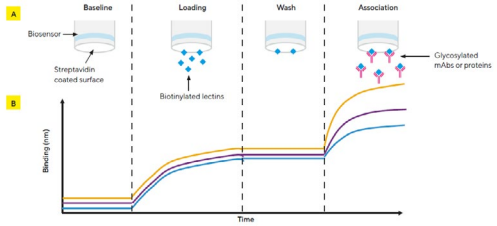

Figure 5: Binding which occurs at each assay step.

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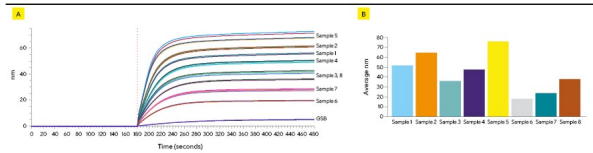

Figure 6: GlyM biosensors binding response.

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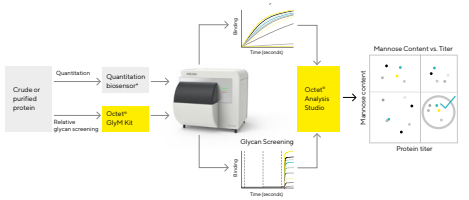


Figure 7: Schematic of mannose screening workflow.

Click to Expand

Post Translational Modification Assessment

Octet® GlyM Kit
The Octet® GlyM Kit includes biosensors preimmobilized with mannose-specific lectin, buffers, and reagents necessary for the detection of mannose in purified and non-purified samples.[15] The lectin used is highly specific towards terminal alpha mannose species, including oligomannose-type N-glycan and hybrid-type N-glycans. It also binds strongly to biantennary complex-type N-glycan, but not tri- and tetra-antennary complex-type N-glycans.

The kit allows for early screening of mannosylation during the subcloning and top clone selection stages of cell line development and upstream bioprocessing and enables users to rank order screened clones based on total mannose content (Figure 6).

With a combination of high-throughput and flexibility of sample analysis using biosensors for titer determination, specificity to target antigen binding and off-rate analysis all from the same sample set, the Octet® platform facilitates early selection of optimal clones with the desired CQAs during cell line development (Figure 7).[16]

Additional Information (click to expand)

- Assay Method
- Octet® GlyM Kit
- Glycosylation CQA for Biosimilars

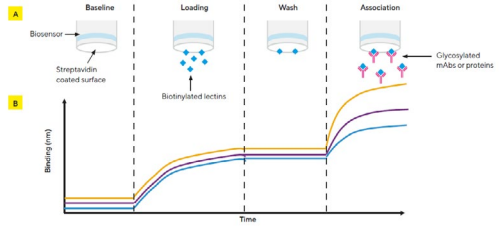


Figure 5: Binding which occurs at each assay step.

Click to Expand

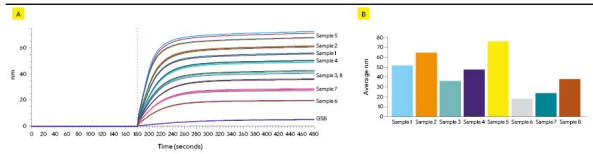


Figure 6: GlyM biosensors binding response.

Click to Expand

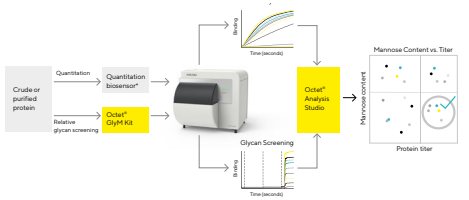


Figure 7: Schematic of mannose screening workflow.

Click to Expand

Post Translational Modification Assessment

Glycosylation CQA for Biosimilars

Glycosylation is also a CQA for biosimilars, with regulatory agencies requiring that heterogeneity be under 5% of the total product volume. The need to minimize batch-to-batch variations supports the application of lectins as an analytical tool because of their high glycan specificity. Fernandez-Poza, et al, described the binding characterization of recombinant prokaryotic lectins (RPLs) displaying specific binding activities to α-mannose, β-galactose, fucose, and sialic acid residues, tested against major biosimilar targets using the Octet® BLI platform.[17] The binding activity of the RPLs and the specificity to a broad range of glycoproteins and glycoconjugates were evaluated and compared to those of equivalent plant-derived lectins. While exhibiting better or similar specificity, RPLs displayed significantly better binding in all cases. The authors note that this enhancement in analytical parameters of RPLs demonstrates their applicability in protein purification and as bioanalytical tools for glycan analysis and biosensor development.

In-process monitoring of glycosylated protein concentration is also essential with the introduction of perfusion bioprocesses. Stantic, et al, described how affinity chromatography based on lectins allows selective monitoring when carbohydrates are accessible on the protein surface.[17] In this study, they immobilized lectin and implemented it for bioprocess monitoring. A spacer was introduced which increased binding kinetics toward an Fc-fusion protein, as assessed using the Octet® platform.

(click to expand)

- Assay Method +
- Octet® GlyM Kit +
- Glycosylation CQA for Biosimilars +

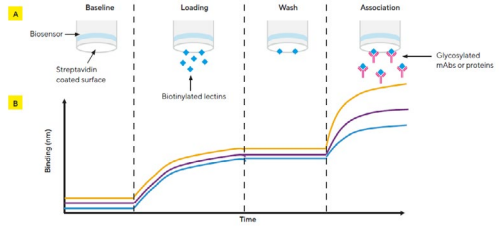


Figure 5: Binding which occurs at each assay step.

+ Click to Expand

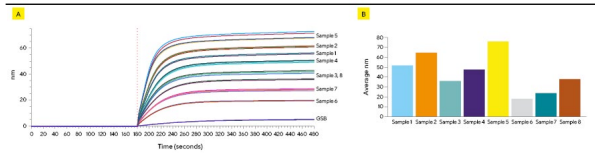


Figure 6: GlyM biosensors binding response.

+ Click to Expand

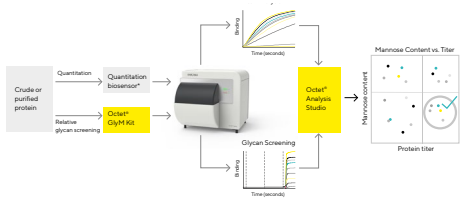


Figure 7: Schematic of mannose screening workflow.

+ Click to Expand

Post Translational Modification Assessment

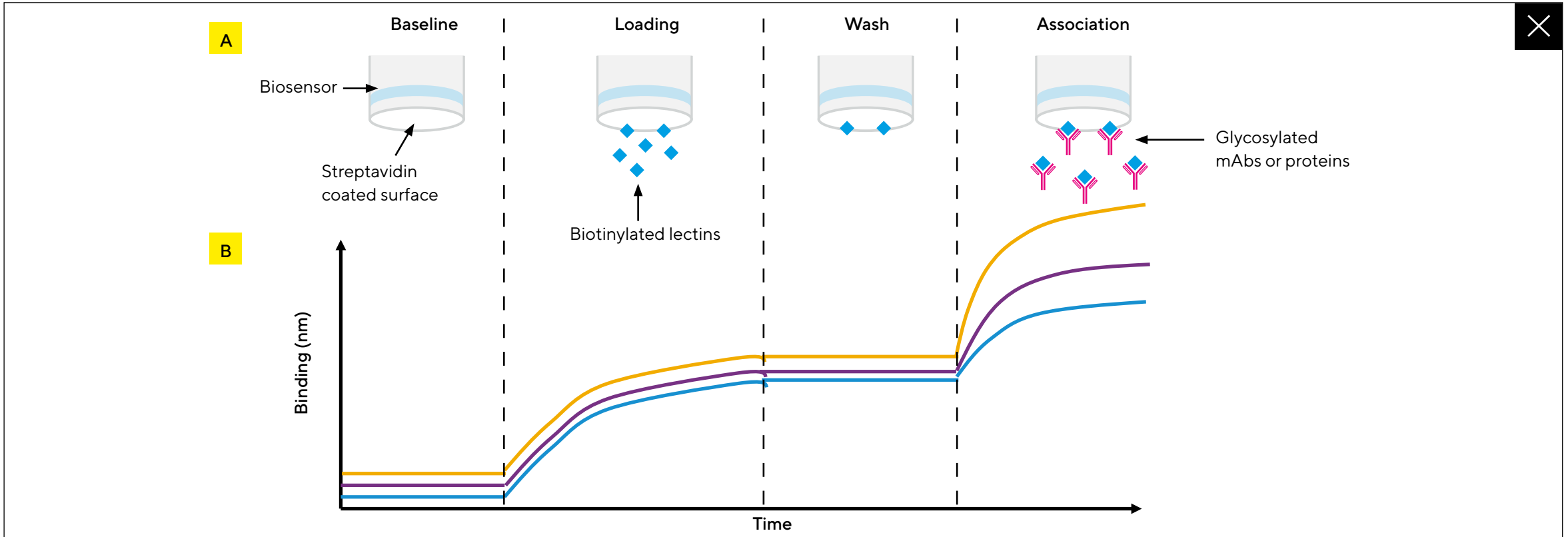
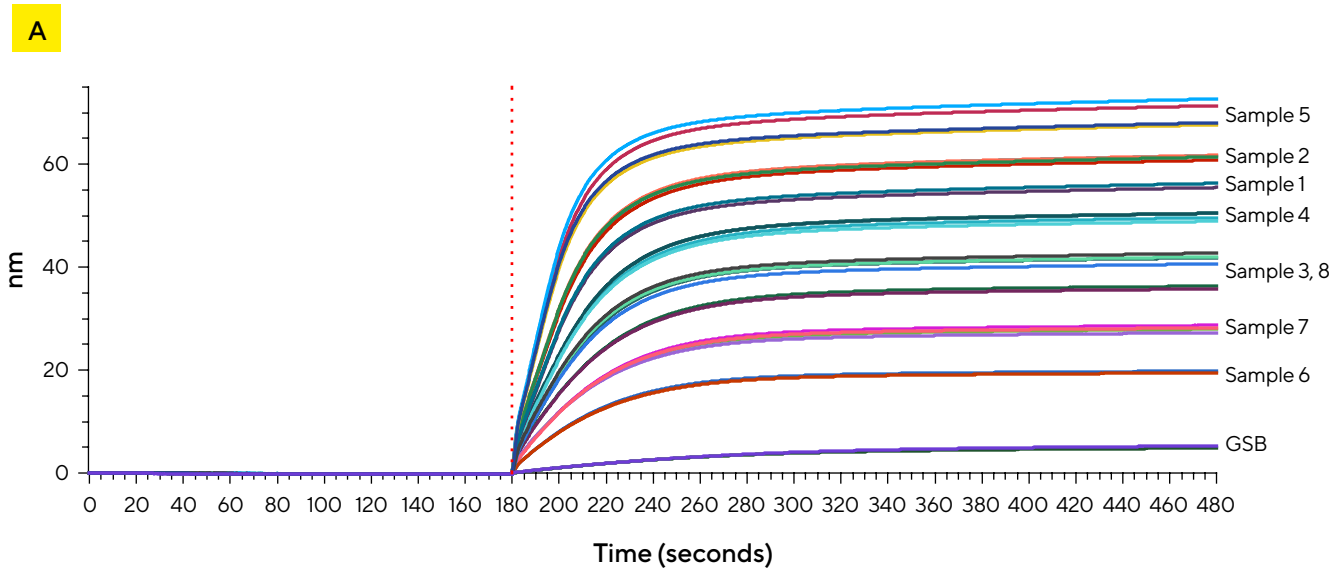


Figure 5: Binding which occurs at each assay step (A) and how this translates to binding (nm) over time (B). The yellow, purple, and blue lines represent high, medium, and low concentrations of glycosylated proteins, respectively.

Post Translational Modification Assessment



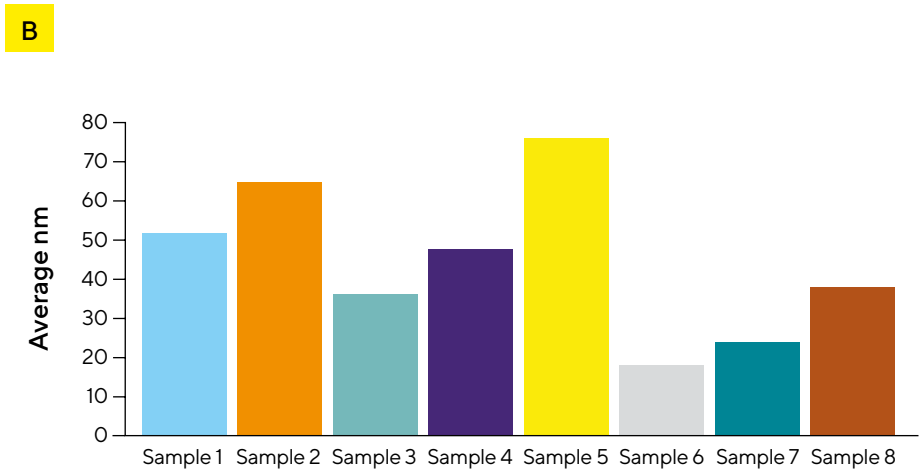


Figure 6: GlyM biosensors binding response signals in a screening assay for multiple human IgG samples with unknown % mannose content (A). An analysis of the binding response magnitude (B) revealed sample 6 to be the most optimal for selection for further development as it exhibits the lowest binding to the biosensors hence indicating presence of the lowest mannose content of the tested samples.

Post Translational Modification Assessment

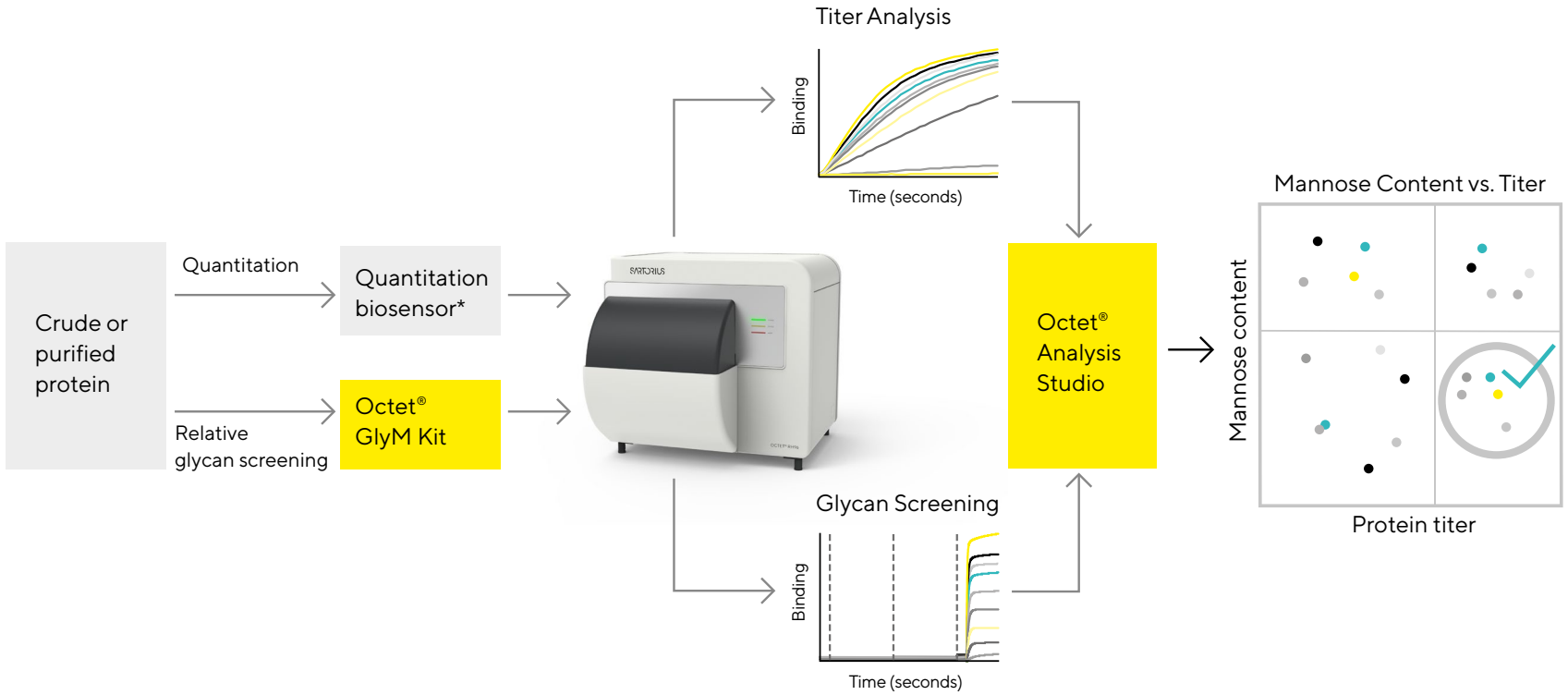


Figure 7: Schematic showing mannose screening workflow using the Octet® GlyM Kit in combination with titer determination to rank the samples by best producer vs. the desired mannose content.

Impurity Testing

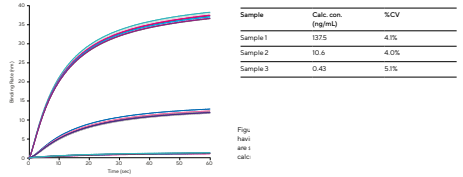
A variety of impurities must be detected and subsequently removed from the biotherapeutic manufacturing workflow; common impurities are Host Cell Proteins (HCP) from cell lines used to produce the drug molecule and residual Protein A which can leach from purification columns. The BLI-based assays offer an accurate, sensitive, and rapid method to detect these contaminants.

An assay for measuring HCPs from Chinese hamster ovary (CHO) cells generated during production of recombinant protein therapeutics can be executed on the Octet® BLI platform.[19] Residual HCPs can reduce the efficacy of a therapeutic and cause adverse immunogenic reactions in patients. Hence, their detection and reduction to the lowest acceptable levels have become critical aspects of drug safety.

While ELISA is commonly used for detection of HCPs, there are several inherent problems with this technique, resulting from its reliance on labor-intensive processing steps that introduce variability, time-consuming incubation steps, and reliance on colorimetric or fluorescent probes that can yield false positive signals. The Octet® BLI platform offers better precision, better or equivalent sensitivity and dynamic range, low user intervention, rapid assay development, and much faster time-to-results when compared with ELISA. The measurement involves a sandwich-type assay on an anti-CHO HCP biosensor coated with anti-CHO HCP antibody.

Additional Information (click to expand)

- Host Cell Protein Detection +
- Residual Protein Detection +



Sample	Calc. con. (ng/mL)	%CV
Sample 1	137.5	4.1%
Sample 2	10.6	4.0%
Sample 3	0.43	5.1%

Figure 8: Data for three unknown samples, each having eight replicates.

Click to Expand

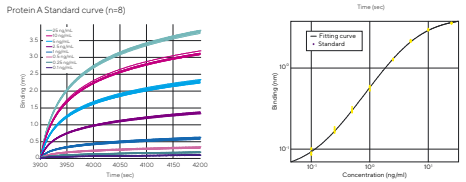


Figure 9: Standard curve made with the residual Protein A detection kit.

Click to Expand

Impurity Testing

Host Cell Protein Detection
A walk-away Host Cell Protein (HCP) assay analyzing 96 samples in parallel can be set up to run automatically on the Octet® RH96 system with results obtained in one hour. Figure 8 shows data for three samples containing unknown concentrations of HCPs, each having eight replicates.

An assay has been implemented by GlaxoSmithKline to streamline detection of HCP in process development.[11] The automated BLI HCP assay required minimal analyst intervention and provided more accurate and precise results than their manual ELISA. Hands-on time for preparation and processing of one to three assay plates was reduced to 30 minutes from the previous 2.5 hours with manual ELISA, and antibody consumption decreased by 40%.

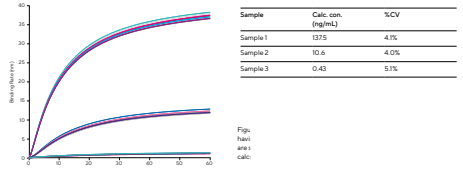


Figure 8: Data for three unknown samples, each having eight replicates.

Click to Expand

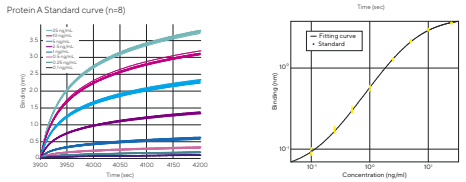


Figure 9: Standard curve made with the residual Protein A detection kit.

Click to Expand

Additional Information (click to expand)

- Host Cell Protein Detection
- Residual Protein Detection

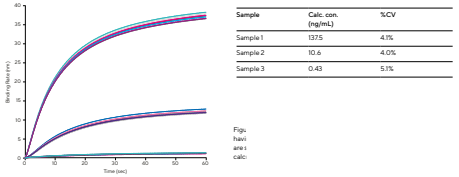
Impurity Testing

Residual Protein Detection
Detection of residual Protein A that has leached off the chromatography matrix and co-elutes with the drug substance is also a critical quality control step in the manufacturing of antibody therapeutics. Testing for leached Protein A is performed in several stages of biologic development and production including purification process development, manufacturing, and finished product release testing.

Residual Protein A detection using a label-free approach can accurately measure down to 100 pg/mL of the contaminant in samples containing up to 5 mg/mL of antibody.[20] The assay uses a validated sample treatment method for dissociating Protein A from antibodies without boiling, neutralization, or centrifugation steps. Following sample treatment, dip-and-read residual protein A biosensors with pre-immobilized chicken anti-Protein A antibody are sequentially dipped into treated samples, buffers, and detection antibody arrayed in 96-well plates to create an immunoassay on the biosensor tip. Signal detection occurs in real time at the secondary antibody step without need for conjugates or enzymatic reactions. Figure 9 shows the standard curve developed using the residual protein A kit in conjunction with the Octet® BLI platform.

Additional Information (click to expand)

- Host Cell Protein Detection +
- Residual Protein Detection +



Sample	Calc. con. (ng/mL)	%CV
Sample 1	137.5	4.1%
Sample 2	10.6	4.0%
Sample 3	0.43	5.1%

Figure 8: Data for three unknown samples, each having eight replicates.

+ **Click to Expand**

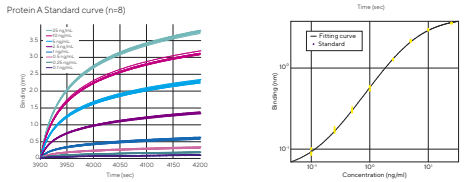


Figure 9: Standard curve made with the residual Protein A detection kit.

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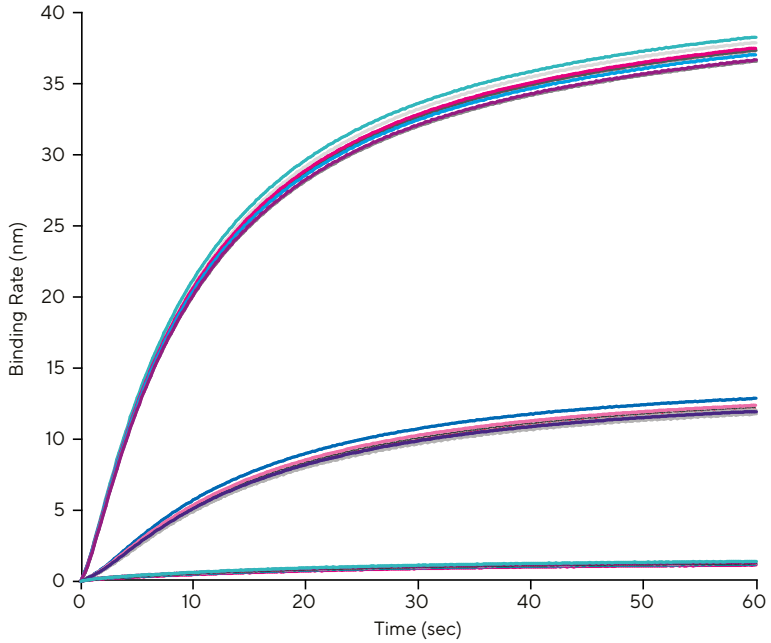
Impurity Testing



Sample	Calc. con. (ng/mL)	%CV
Sample 1	137.5	4.1%
Sample 2	10.6	4.0%
Sample 3	0.43	5.1%

Figure 8: Graph showing data for three unknown samples, each having eight replicates. The calculated concentrations of HCP and %CV are shown in the accompanying table.

Impurity Testing

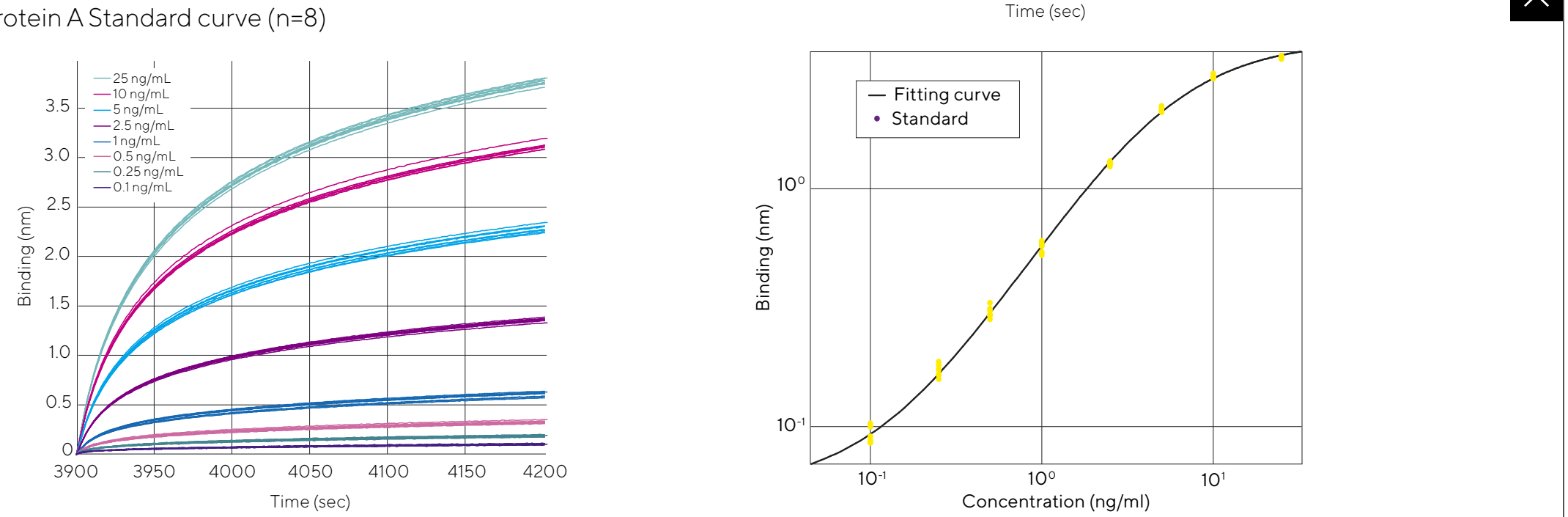


Figure 9: Standard curve made with the residual Protein A detection kit standard.

Concentration and Potency Determination

The concentration of a biotherapeutic molecule in a sample can be rapidly determined by a direct binding or sandwich assay on the Octet® BLI platform (Figure 10).[11,21] In a typical quantitation assay, a standard curve is generated, and unknown sample concentrations are interpolated. Concentration can be calculated from the interaction's initial binding rate, which is based on the initial slope or from the binding rate at equilibrium.

This approach was used by Boehringer Ingelheim to develop an assay to measure antibody Fab concentration and activity.[22] Relative to the overnight incubation and four-hour assay time of a conventional ELISA protocol, the assay provided an analysis time of only one hour per 96-well microplate, including sample preparation time. This assay was used to monitor Fab activity for all process development studies and was subsequently qualified for use in quality control. Drug activity measurement using the Octet® BLI platform has become a critical parameter for their product evaluation and has resulted in increased Fab drug product consistency and quality.

Additional Information (click to expand)

- Functional Biological Activity
- Vaccine Titers

Figure 10: Comparison of protein quantitation in complex matrices using Octet® BLI systems and alternative methods.

Click to Expand

Concentration and Potency Determination

Functional Biological Activity

Functional biological activity is a CQA essential to verifying the potency of a drug molecule. Potency assays can be used throughout the development process in comparability and formulation studies and are required for release of every lot of therapeutic protein. Cameron, et al, outlined strategies for the development and validation of a potency assay using Octet® BLI systems using the binding of an Fc gamma receptor III (FcγRIIIa) molecule to the widely characterized NIST mAb as a model system.[23] The authors describe considerations for the development of a percentage relative potency method for FcγRIIIa that was capable of early-phase comparability studies and subsequent method validation for lot release. With the speed of the Octet® system, they rapidly developed Design of Experiment results which led to development, optimization, and potential validation practices.

Silva and Dass described assay optimization on the Octet® BLI platform for comparability binding studies during drug development at a contract bioanalytical testing laboratory.[24] The high throughput capabilities of the system enabled assay conditions to be established in a fraction of the time needed using conventional binding assays. The platform was used for a binding kinetics assay for a product comprising three types of monoclonal antibodies expressed using the same host cell line, optimization of assays to compare the binding kinetics and specificity of an originator and two biosimilar antibodies to macrophage mannose receptors, and compare the binding characteristics of one originator and two biosimilar antibodies for VEGF-A isoform 165.

Figure 10: Comparison of protein quantitation in complex matrices using Octet® BLI systems and alternative methods.

Click to Expand

Concentration and Potency Determination

Vaccine Titers

The Octet® BLI platform can also be used to rapidly determine vaccine titers during the manufacturing process. Historically, the single radial immunodiffusion (SRID) technique has been used for vaccine titer determination. However, SRID is time consuming and generally exhibits poor precision. In contrast, the Octet® platform reduces assay time from days to just a few hours for a 96-well plate of samples. Wheatley, et al, describe development of an influenza vaccine titer assay with an improved relative standard deviation and dynamic range compared to SRID.[25]

The assay is based on the binding of the vaccine to polyclonal antibodies that recognize the influenza epitopes presented by the vaccine. The assay format is suitable for detecting the rapid changes in the viral strains represented in a vaccine; rapid changes between vaccines derived from different viral strains are enabled by simply binding the paired antibody for the new strain to a biosensor without the need for derivatization. Unlike the SRID technique where detergents are used to expose the target hemagglutinin molecule, with Octet® BLI systems, samples are analyzed in their natural state without the use of detergents. As a result, Octet® BLI systems can analyze whole virus, split virions and recombinant HA vaccine samples.

Figure 10: Comparison of protein quantitation in complex matrices using Octet® BLI systems and alternative methods.

Click to Expand

Concentration and Potency Determination

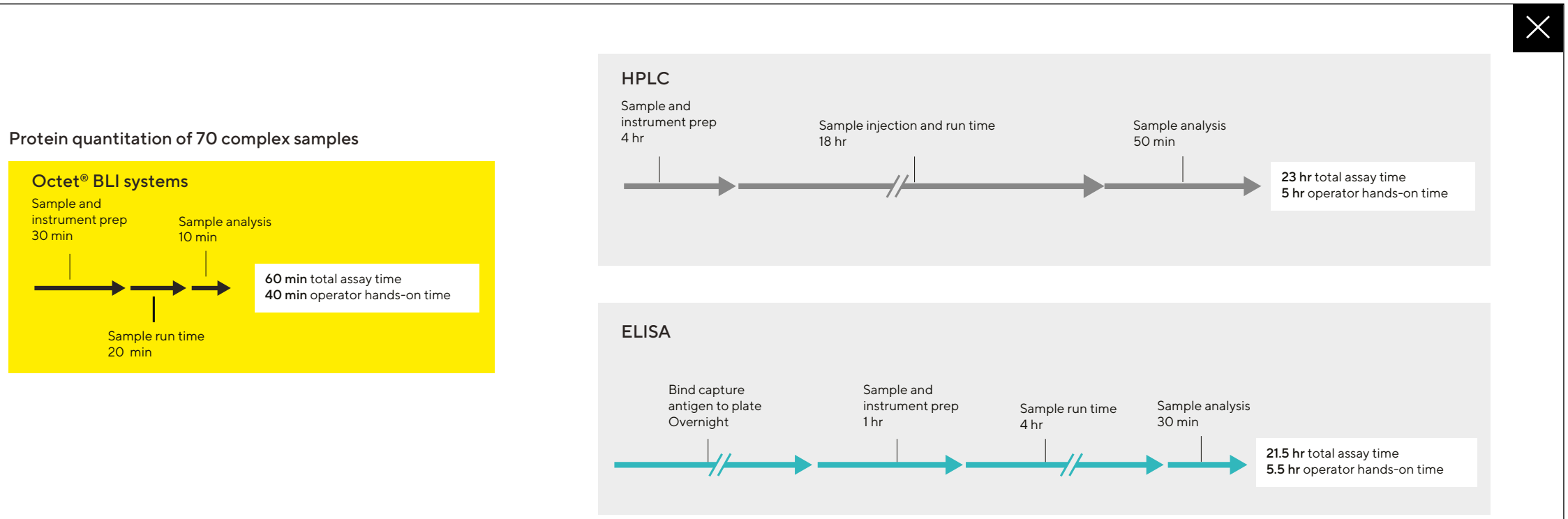


Figure 10: Comparing protein quantitation in complex matrices using Octet® BLI systems, HPLC or ELISA..

EC_{50} and IC_{50} Studies

A potency or potency-indicating assay is required regulatory bodies prior to the release of every manufactured drug lot. The Octet® BLI platform can be used for the measurement of EC_{50} and IC_{50} when drug binding activities to target molecules correlate with the biological activity, stability, or quality attributes such as glycosylation. The EC_{50} is the concentration of a drug that gives half-maximal response, while the IC_{50} is the concentration of an inhibitor where the response (or binding) is reduced by half. These dose-response assays provide a rapid time to results with automated workflows, minimal hands-on times, and reduced assay variabilities. Unlike ELISA-based potency assays, all assay steps can be monitored and evaluated in real-time.

Cai, et al, described use of the Octet® platform to measure the binding of HIV-1 antigen and CAP256V2LS, a broadly neutralizing monoclonal antibody being pursued as a promising drug for HIV-1 prevention.[26] Binding of CAP256V2LS that had varying levels of tyrosine sulfation to native like HIV-1 Env protein was used to measure how tyrosine sulfation levels affected binding potencies. CAP256V2LS was bound to a Protein A biosensor which was then washed to remove unbound antibody. The sensor-antibody complex was then dipped in a well containing HIV-1 antigen. Binding results for the CAP256V2LS- HIV-1 antigen interaction was measured on an Octet® BLI platform and given as nanometer shift. Increases in bound CAP256V2LS- HIV-1 antigen complexes result in greater wavelength shifts; the antibody-antigen binding curve was obtained by plotting the wavelength shifts versus the antibody concentration.

Application Note: A Simple Method to Determine Relative Potency (EC_{50})

Datasheet: Octet® Software Version 13 | Added Features to Improve EC50, IC50 and Potency Analysis

Stability Studies

Octet® BLI systems can be used for stability or potency indicating studies and are suitable for measuring and distinguishing fully functional drug products and those whose binding activities have been affected by degradation.[26, 27] While other biophysical analytical tools are available for detecting aggregates, the Octet® platform offers the advantage of also performing binding analyses in tandem.

A hydrophobic-based probe can be used to screen for and to evaluate molecular structure including unfolded proteins and differentiate between pre-aggregates and aggregate formation. In addition, the plate design, and the use of multiple biosensors in parallel enables screening of buffers or formulations to help determine the optimal formulation conditions. Figure 11 demonstrates the ability of a method developed on the Octet® BLI platform to rapidly detect pre-aggregate/aggregate formation in protein solutions during slightly elevated (40–45°C) to moderate (55°C) thermal stress.

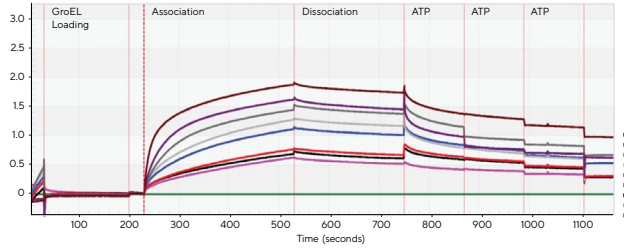


Figure 11: Monitoring the evolution of polyclonal IgG binding amplitude with increasing temperature.

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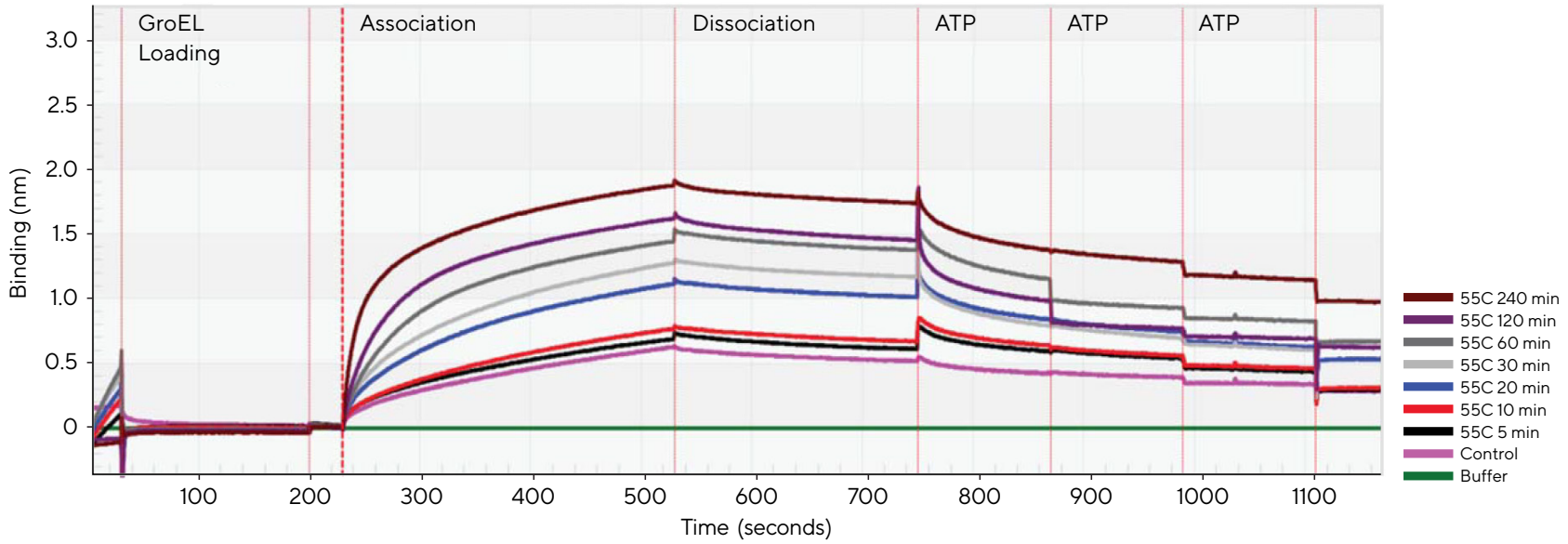
Stability Studies



Find out more: Probing structurally altered and aggregated states of therapeutically relevant proteins using GroEL coupled to bio-layer interferometry

Figure 11: Monitoring the evolution of polyclonal IgG binding amplitude with increasing temperature.[28] Data courtesy of University of Kansas.

Conclusion

As their name implies, critical quality attributes are essential contributors to the efficacy and safety of therapeutics; they must be defined and monitored throughout the drug development process. By eliminating the need for reagent labeling while allowing for real time visualization of binding data across a wide variety of assays, the Octet® BLI platform reduces CQA assay development time and enables greater speed, flexibility and efficiency throughout manufacturing and quality control workflows.

Application Note (click to view document)	Binding Kinetics	Concentration and Titer	Impurity Testing	Validation Studies	Activity Assays	Aggregation and Protein Stability	Lot Release
Biomolecular Binding Kinetic Assays on the Octet® BLI Platform	■						
Enhancing Efficiency and Economics in Process Development and Manufacturing of Biotherapeutic		■		■			■
CHO Host Cell Protein Detection			■				
Residual Protein A Detection Kit			■				
Octet® Potency Assay: Development, Qualification and Validation Strategies				■	■		
Validated Quantitation and Activity Assay of Antibody Fragment Molecule (Fab) for Process Development and Quality Control				■	■	■	■
Octet® BLI Systems: Modernize Biopharmaceutical QC Testing to Increase Efficiency					■		■
A Fast and High Precision Influenza Vaccine Potency Assay					■		
Expanding Octet® Applications in Downstream Biologics Characterization: Stability, Formulations, and Aggregation Studies						■	
Enhanced Productivity and Labor Efficiency in Lot Release and In-Process Testing of Biologics							■

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References

1. ICH Q8 (R2) Pharmaceutical development. 2009.
2. Arpiyo, D. Accelerating the development and manufacture of therapeutics using the Octet platform. Bioprocess International. September 2021.
3. Simplifying biotherapeutic manufacturing and quality control with label-free biosensor technology. Sartorius Application Note.
4. Tang, X-C., Agnihothram, S.S, Jiao, Y., Stanhope, J., Graham R.L., Peterson, E.C., Avnir Y., St. Clair Tallarico, A., Sheehan, J., Zhu, Q., Baric, R.S., Marasco, W.A. Identification of human neutralizing antibodies against MERS-CoV and their role in virus adaptive evolution. PNAS, 111 (19) E2018-E2026. https://doi.org/10.1073/pnas.1402074111.
5. Tsuji I., Dominguez D., Egan M.A., Dean H.J. Development of a novel assay to assess the avidity of dengue virus-specific antibodies elicited in response to a tetravalent dengue vaccine. J Infect Dis. 2022 May 4;225(9):1533-1544. doi: 10.1093/infdis/jiab064. PMID: 33534885; PMCID: PMC9071338.
6. Tobias, R., Yao, D., Kumaraswamy, S. Analysis of Fc-gamma receptor-IgG interactions on the Octet® platform. Sartorius Application Note.
7. Geuijen, K.P.M., Oppers-Tiemissen, C., Egging, D.F., Simons, P.J., Boon, L., Schasfoort, R.B.M. and Eppink, M.H.M. (2017), Rapid screening of IgG quality attributes – effects on Fc receptor binding. FEBS Open Bio, 7: 1557-1574. https://doi.org/10.1002/2211-5463.12283.
8. Label-free technologies for accurate determination of kinetics and affinity rate constants. Sartorius Application Note.
9. Li, H. Cell line development: Accelerating antibody discovery by monitoring titer and glycosylation with the Octet® platform. Sartorius Application Note.
10. Li, H., Kumaraswamy, S., Rubio-Marrero, E., Huang, D., Pendse, G. A rapid method to quantitatively screen bispecific antibodies using Protein A and Octet® His1K biosensors. Sartorius Application Note.
11. Takkar, R., Kumaraswamy, S. Enhancing Efficiency and Economics In Process Development and Manufacturing of Biotherapeutics. Sartorius Application Note.

References

12. Kirby, J., Rees-Manley, A., Apiyo, D., Klein, L., Diem, E., Dale, T. Cell line development: Accelerating process optimization by combining Ambr® 15 cell culture with Octet® titer measurements. Sartorius Application Note.
13. Smith, V., Wharram, L., Jamieson, S. Innovations in analytical science: development of rapid, high-throughput glycoanalytics for biopharmaceuticals. European Biopharmaceutical Review. https://www.europeanpharmaceuticalreview.com/article/137002/innovations-in-analytical-science-development-of-rapid-high-throughput-glycoanalytics-for-biopharmaceuticals/
14. Webinar: Novel high-throughput glycoanalytics for biopharmaceuticals. https://www.sartorius.com/en/pr/octet/novel-high-throughput-glycoanalytics-for-biopharmaceuticals-webinar
15. Octet® GlyM kit for high-throughput mannose glycans screening of crude and purified mAb and non-mAb protein samples. Sartorius Technical Note.
16. Krylov, I., Marusic, J., Zhang, Y., Murphy, A., Zhang, L., Apiyo, D. Mannose glycans content screening for human-mAb samples using the Octet® GlyM kit. Sartorius Application Note.
17. Fernandez-Poza, S., Padros, A., Thompson, R., Butler, L., Islam, M., Mosely, J.A., Scrivens J.H., Rehman, M.F., Muhammad, Akram, M.S. Tailor-made recombinant prokaryotic lectins for characterization of glycoproteins, Analytica Chimica Acta, Volume 1155, 2021, 338352. https://doi.org/10.1016/j.aca.2021.338352
18. Stantič, M., Gunčar, G., Kuzman, D., Mravljak, R., Cvijić, T., Podgornik, A. Application of lectin immobilized on polyHIPE monoliths for bioprocess monitoring of glycosylated proteins, Journal of Chromatography B, Volume 1174, 2021, 122731. https://doi.org/10.1016/j.jchromb.2021.122731.
19. CHO host cell protein detection. Sartorius Technical Note.
20. Residual Protein A detection kit. Ready BLI kit for simple, sensitive, and accurate quantitation of leached Protein A in bioprocess samples. Sartorius Technical Note.
21. Apiyo, D. Accelerating the development and manufacture of therapeutics using the Octet platform. Bioprocess International. September 2021.

References

22. Takkar, R., Kumaraswamy, S., Zaremba, S. Validated quantitation and activity assay of antibody fragment molecule (Fab) for process development and quality control. Sartorius Application Note.
23. Cameron, C., Peacor, B., Oien, N., Cheeseman, A., Smedley, J., Laughlin, J., Apiyo, D. Octet® potency assay: Development, qualification, and validation strategies. Sartorius Application Note.
24. Webinar: Utilizing Octet BLI platform to characterize large molecule critical reagents in support of drug development for contemporary biotherapeutics.
25. Wheatley, D., Saunders, D., Jones, K., Apiyo, D. A fast and high precision influenza vaccine potency assay.
26. Cai, C.X., Doria-Rose, N.A., Schneck, N.A. et al. Tyrosine O-sulfation proteoforms affect HIV-1 monoclonal antibody potency. Sci Rep 12, 8433 (2022). https://doi.org/10.1038/s41598-022-12423-x.
27. Apiyo, D. Expanding Octet® applications in downstream biologics characterization: Stability, formulations and aggregation studies.
28. Naik, Subhashchandra et al. Probing structurally altered and aggregated states of therapeutically relevant proteins using GroEL coupled to bio-layer interferometry. Protein science: a publication of the Protein Society vol. 23, 10 (2014): 1461-78. doi:10.1002/pro.2515.

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