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Octet® ProA-MAX Biosensors for High Titer IgG Quantitation

Comparable Performance with Protein A HPLC

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Abstract

The increasing adoption of monoclonal antibodies (mAbs) as therapeutic products requires continuous improvement and optimization of their development and manufacturing processes. Monitoring mAb titer in cell culture during both the cell line development (CLD) and bioreactor stages is essential not only for rapidly identifying cell lines with high expression levels, but also for determining optimal bioprocess conditions for mAb expression. The dynamic range, accuracy, and consistency are critical attributes of any analytical technology used for mAb quantification. This white paper compares the performance of Octet® Pro-MAX Biosensor with Protein A HPLC for mAb titer measurement and outlines the benefits of each method.

The Octet® Bio-Layer Interferometry (BLI) platform with Octet® ProA (Protein A) Biosensors has gained prominence over the years as a go-to technology for rapid, high-throughput titer determination of mAbs and Fc-fusion proteins in both crude and purified samples. In the workflow, Octet® ProA Biosensors are dipped directly into samples, and binding of the target protein to the biosensor surface is monitored in real time. Because the binding rate is proportional to protein concentration, measurements from reference samples with known titers are used to generate a standard curve, which is then used to determine titers in unknown samples. Besides the high-throughput, the key advantages of the Octet® BLI platform include ease of use, robustness, and low maintenance costs. In addition, the biosensors can be reused multiple times to signficantly reduce the cost of analysis per sample. To date, one limitation of the Octet® ProA Biosensor has been its narrower dynamic range of 0.025–2000 µg/mL, which requires serial dilution for samples with higher titers and increases both analysis complexity and time to results. To address this limitation, Octet® ProA-MAX Biosensors offer an extended dynamic range of 100–12000 µg/mL, enabling direct quantification of samples within this titer range without serial dilution while maintaining the high precision and accuracy associated with Octet® ProA Biosensors.

To demonstrate the versatility of the Octet® ProA-MAX Biosensor, its performance was compared with widely used Protein A-based high-performance liquid chromatography (HPLC). Because HPLC offers a broad dynamic range, high precision, and high accuracy, it serves as a strong benchmark for evaluating new mAb quantification platforms. The side-by-side comparisons of key sample processing and assay parameters showed similar performance between Octet® ProA-MAX and HPLC, as summarized in Table 1.

Samples Processing and Assays Run Parameters

	Protein A HPLC Method	Octet® ProA-MAX Biosensors
Column Equilibration & Valve Setup	Yes	n.a
Sample Dilution	No	No*
Sample Filtration	Yes*	n.a.
Plate Preparation	n.a.	5 min
Vial Transfer	Yes	n.a.
Biosensor Hydration	n.a.	10 min
Time to analyze 96 samples	192–2400 min**	Octet® R8e/R8: < 30 min Octet® RH16: < 15 min Octet® RH96: 2 min

Table 1. A comparison of assay processing steps and samples detection time between Protein A HPLC and Octet® ProA-MAX Biosensors. The sample detection time represents the estimated assays run time for 96 mAb samples for three different Octet® configurations when compared to the same number of samples for HPLC.

* Sample dilution as a function of matrix may be necessary on the Octet® (see the Octet® ProA-MAX Technical Note) while sample clarification may be needed on HPLC for crude samples.

** Approximately 2–25 min/sample, depending on the method and column used[1,2]

Protein A HPLC and Octet® ProA-MAX Calibration Curves for hIgG Titer Quantification

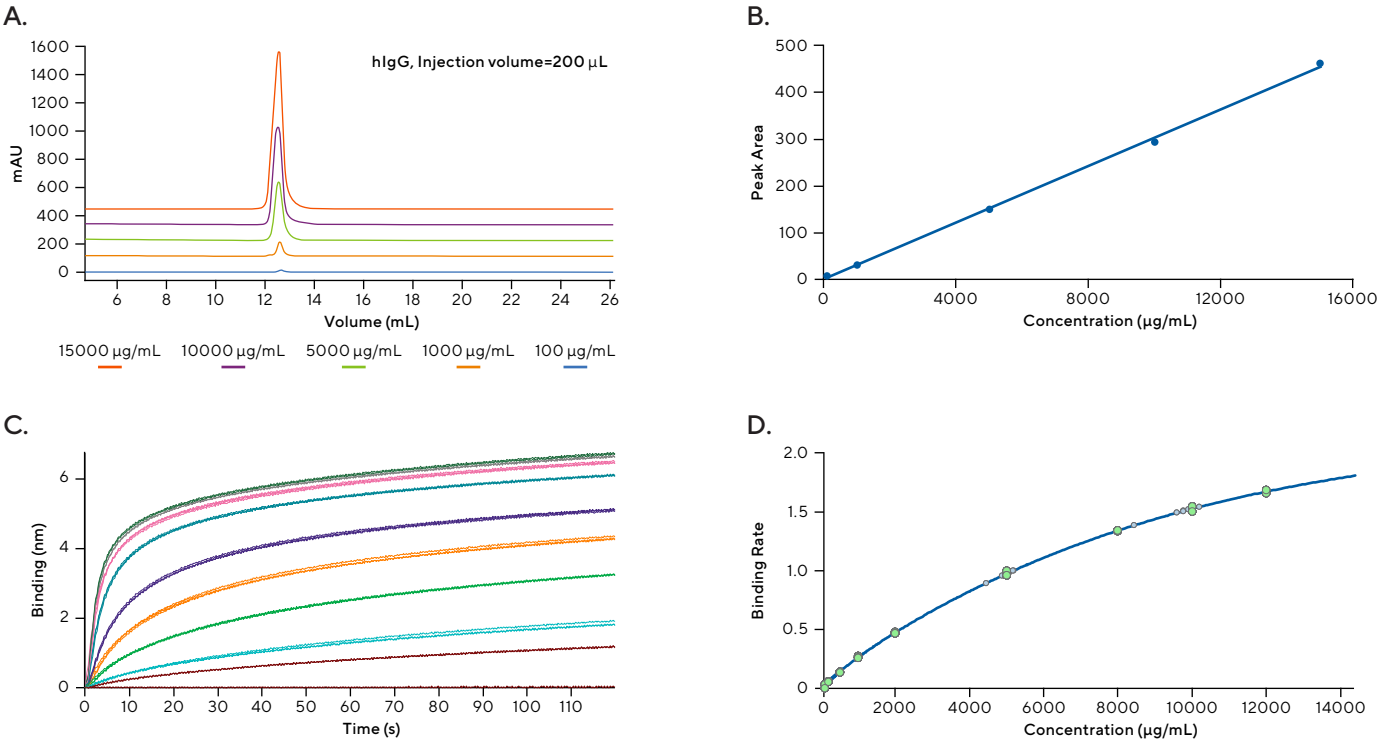

Figure 1. hIgG standard calibration curves generated using Protein A HPLC (A, B) and using Octet® BLI Platform and ProA-MAX Biosensors (C, D). The ProA-MAX Biosensor calibrator curve was generated from triplicate samples with titers in the range 100–12000 µg/mL and acquired at the shake speed of 400 RPM. HPLC data was generated from triplicate samples with titers in the range 100–15000 µg/mL and acquired at a Flow rate of 1.0 mL/min.

Protein A HPLC—Octet® ProA-MAX Biosensor Performance Comparison

To evaluate and compare the performance of Octet® ProA-MAX Biosensors and HPLC, hIgG standard calibration curves spanning a titer range of 100–12000 µg/mL were generated on both platforms (Figure 1). hIgG test samples with titers ranging from 200 to 10,000 µg/mL were then measured on each platform and analyzed using the corresponding calibration curves. Correlation of data between the two was found to be strong with an R^2=0.99 for the sample titer range tested (Table 2 and Figure 2). Slight deviation from nominal concentration was observed for both techniques, with the biosensors exhibiting a slightly negative bias while HPLC exhibited a slightly positive bias. This differing trend is likely attributed to the inherent differences between the active (Protein A) molecules between the HPLC resin and the biosensor surface.

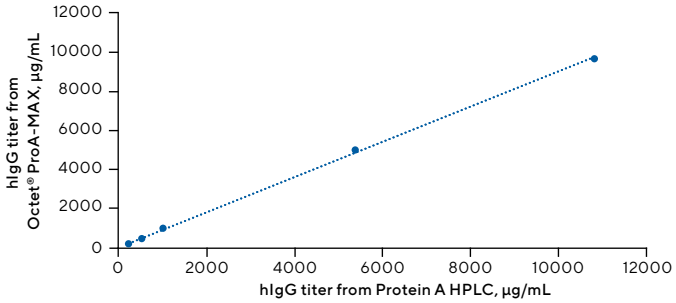

Figure 2. Protein A HPLC and Octet® ProA-MAX Biosensor exhibit a strong correlation in mAb detection across the range of 100–12000 µg/mL with an R^2=0.99.

Known hIgG Titer, µg/mL	Protein A HPLC			Octet® ProA-MAX Biosensor		
	Average Measured hIgG Titer, µg/mL (n=3)	% Recovery	% Bias	Average Measured hIgG Titer, µg/mL (n=3)	% Recovery	% Bias
200	227.4	113.7	13.7	198.1	99.05	-0.95
500	517.6	103.5	3.52	467.6	93.5	-6.48
1000	999.4	99.9	-0.06	983.5	98.3	-1.65
5000	5381.7	107.6	7.63	5017.2	100.3	0.34
10000	10809	108.1	8.09	9663.4	96.6	-3.37

Table 2. Accuracy and detection bias comparison between the Octet® ProA-MAX Biosensors and HPLC. The two platforms correlate well albeit with some bias observed.

Summary

Octet® ProA Biosensors offer a great solution for mAb titer measurement and meet user requirements for precision and accuracy[3] at all stages of mAb development; however, their dynamic range (0.025–2000 µg/mL) necessitates serial dilution of samples when the expected mAb titer is greater than 2000 µg/mL. Octet® ProA-MAX Biosensors overcome this limitation by expanding the dynamic range to 100–12000 µg/mL and eliminating the need for serial dilution, thereby simplifying the workflow and reducing time to results for mAb quantification in high titer samples.

Compared to Protein A HPLC, Octet® ProA-MAX Biosensors provide comparable precision and accuracy while enabling rapid, high-throughput high titer analysis that can be implemented earlier in the mAb development process.[4] Furthermore, the Octet® method offers two major advantages over HPLC: reduced assay costs and shorter overall analysis time, which may in turn lower FTE-related costs.[5]

References

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