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Developing a Robust Quantitation Assay for Monoclonal Antibodies and Other Proteins on the Octet® Platform

Rashi Takkar, Applications Scientist, rashi_takkar@pall.com; Sriram Kumaraswamy, Director, Marketing Field Applications, sriram_kumaraswamy@pall.com

INTRODUCTION

The Octet platform provides analytical methods for measuring antibody and protein concentrations and binding affinities through various stages of drug discovery and development (Table 1). Octet systems circumvent many limitations of ELISA and HPLC assays, enabling informed decisions to be made earlier in both drug and process development – particularly when chromatography and other critical separation steps are needed.

Octet systems perform automated sample analysis in 96- and 384-well microplate formats. The simple Dip and Read™ approach enables streamlined workflows and rapid quantitation of 96 samples in as little as 20 minutes, or 384 samples in 70 minutes. In a typical quantitation assay, biosensors coated with capture molecules dip into samples and the concentration of the target

Phase	Group	Function	Octet Value to User
Research & Development	Hybridoma or Phage Screening	Protein or antibody quantitation in hybridoma supernatants or lysates (for affinity screen and to normalize functional activity).	High throughput, faster time to result, compatible with crude matrices (no dilution needed).
	Post-screening	1) Mammalian clone selection based on titers in transients: 50–300 µg/mL (supernatant) and stables: 100–1000 µg/mL (bioreactors). 2) Determine concentration to load on HPLC for small-scale purification.	
Upstream Process Development	Cell Culture	Determine best growth conditions: media, growth supplements, vectors, etc. Mammalian clone selection based on expression. Stables: 1–300 µg/mL (supernatant).	Easy to use, high throughput, low running costs per sample, faster assay development and time to result compared to HPLC and ELISA, higher precision than ELISA, compatible with crude matrices so no sample prep needed, integration with liquid handling platforms.
	Cell Culture Scale-up	Select promising clones to scale-up for downstream clinical studies. Stables: 1–10 mg/mL (bioreactors).	
Downstream Process Development	Purification	Antibody or protein quantitation following different stages of purification.	Measures active rather than total protein (i.e., A280), easy to use, faster assay development and time to results compared to HPLC, GMP compatible, robust, accurate, low running costs.
	Impurity Analysis	Measure trace levels of Host Cell Protein or Residual Protein A.	Fast assay development and time to result, sensitive, automated assay format, GMP compatible.
Quality Control & Manufacturing	QC Analytical Group	Quantitation of drug molecules.	Measures active protein, provides binding activity data, label-free analysis, GMP compatible, robust and accurate.

TABLE 1: Antibody quantitation in biopharmaceutical development and the Octet system's role at each stage.

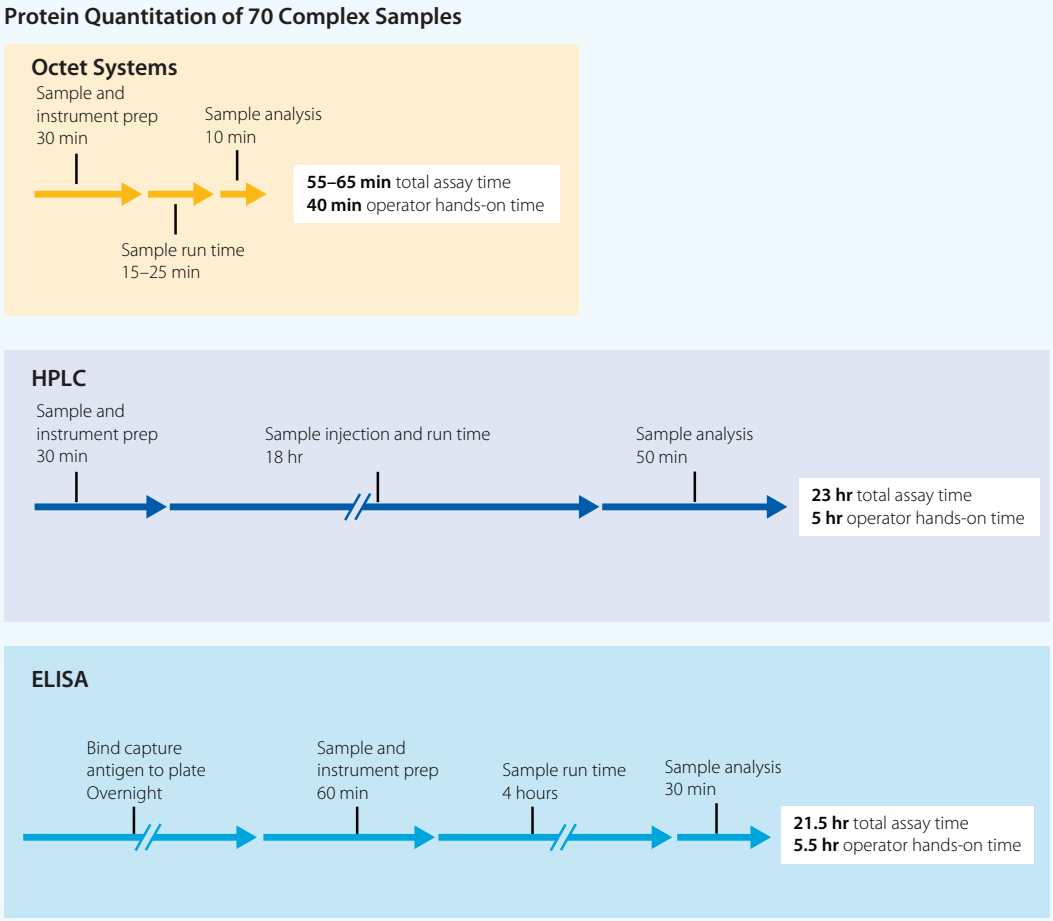

FIGURE 1: Comparison of time and effort involved in protein quantitation using the Octet platform and alternative methods.

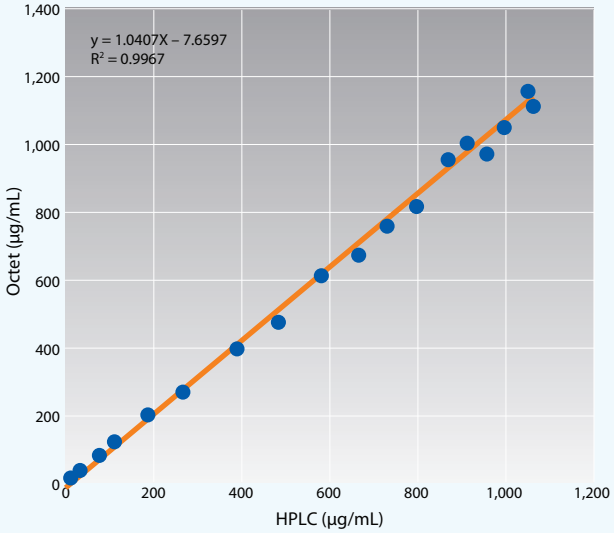

FIGURE 2: Excellent correlation of Octet platform data with HPLC data for human IgG concentrations over a broad dynamic range.

protein is determined. A standard curve is generated using known amounts of the target, and concentrations of unknown samples are extrapolated from the standard curve.

ACCURACY OF OCTET PLATFORM DATA COMPARED TO HPLC

As shown in Figure 2, Octet platform concentration data have been shown to compare well to values determined by standard techniques such as HPLC. Antibody quantitation on Octet systems is accurate and precise over a broad dynamic range of concentrations, even in crude samples.

ASSAY COSTS COMPARED TO ELISA AND HPLC

Comparison of time and effort involved in generating results (Figure 1) shows that the labor and time savings on the Octet platform leads to lower cost per sample in comparison to ELISA and HPLC. The low cost, single use biosensors circumvent the need for reuse and revalidation. However, many biosensors can be regenerated and reused multiple times if needed, enabling even greater cost-per-test savings.

INSTRUMENT SELECTION

Using Protein A biosensors, the Octet RED96 and RED384 instruments provide a large dynamic range of detection for human IgG from 0.050–2000 µg/mL, whereas the Octet QK^{e} and QK384 systems detect concentrations from 0.1–700 µg/mL. For the highest accuracy and precision, it is recommended to work in the linear section of the standard curve.

QUANTITATION-QUALIFIED BIOSENSOR SELECTION

Target protein quantitation is possible for a number of different types of analyte, using biosensors shown below.

- Anti-Human IgG Quantitation (AHQ)
- Anti-Murine IgG Quantitation (AMQ)
- Anti-FLAG (FLG)
- Protein A (ProA)
- Protein G (ProG)
- Protein L (ProL)
- Anti-Penta-His (HIS)
- Streptavidin (SA)
- Anti-Human Fab-CH1 (FAB)
- Anti-GST (GST)
- Ni-NTA (NTA)

These biosensors exhibit high specificity and are designed to perform titer measurements in the presence of host cell proteins, simplifying analysis at all stages of development. For more information on each biosensor, please refer to the biosensor data sheets at the Pall ForteBio website.

TIPS FOR DEVELOPING AND VALIDATING AN OCTET QUANTITATION ASSAY

Developing an Octet platform assay for in-process testing is both rapid and simple. Octet system users highly value the reduced number of sample preparation and assay steps. For example, not having to purify samples saves considerable time and greatly improves the accuracy and precision of assay results.

Some best practices for developing a quantitation assay on Octet systems for analyte titer determination are listed below:

1. Analyte (antibody) binding to capture molecules like Protein A, G or L on the biosensor is dependent on species and isotype among other factors, so ensure that standards are appropriate for the samples being tested.
2. Determine the useful standard curve concentration range for the analyte, and work in the linear section of the standard curve.
3. The default settings of 400 RPM shake speed for the sample microplate, with a 2-minute read time per sample column, are suitable for working at the higher end of the concentration dynamic range (range is dependent on the type of Octet system, choice of biosensor, sample matrix and the analyte). If higher sensitivity is desired, the shake speed and read time should be increased to 1000 RPM and 5 minutes, respectively.
4. Standard samples must be diluted in the same buffer matrix as the unknown samples.
5. During standard curve generation and testing of unknown samples, run a reference sample to check for non-specific binding, and perform reference subtraction. The reference sample is ideally one that has no analyte and matches the matrix of the unknown samples.
6. Test accuracy of the assay by measuring samples where known amounts of purified analyte are spiked into the matrix. The recovery values should fall within an acceptable range.
7. The samples being analyzed may be run neat or diluted, as appropriate, to bring the concentration within the linear dynamic range of the standard curve.
8. Intermediate precision of the assay may be validated by running several spiked samples in replicates on different days by various operators.
9. Evaluate assay specificity for the antibody of interest by spiking similar antibodies in the matrix. Spiking should not alter assay signal of the target antibody.
10. Standard samples may be run in duplicate or triplicate. Some users average eight replicates of each standard concentration the Octet system (eight wells in one column of a 96-well microplate) to construct a 10-12 point standard curve.
11. When transferring an HPLC assay to an Octet system, ensure that pre-processing is consistent for samples tested with both

techniques. With some samples, preparation steps such as centrifugation at high speeds and/or filtering could result in sample loss that might affect correlation of results between the two techniques.

12 UV monitoring at 214 nm via HPLC is performed on dilute samples (1-300 µg/mL), and this may offer a more direct comparison to values obtained on the Octet systems. UV monitoring at 280 nm on the HPLC is performed on more concentrated samples.

13 In Octet data analysis, previously saved standard curves can be imported into new data sets. The accuracy of calculated concentrations using saved standard curves should be validated against freshly generated standard curves.

14 To re-use biosensors via regeneration, perform scouting experiments to find an optimal regeneration buffer and condition. Ensure that biosensors are preconditioned to obtain the best consistency between different regeneration cycles. For more specific information on regeneration of biosensors, please refer to the appropriate data sheet at the Pall ForteBio website.

GMP OPERATION

The Octet platform includes both IQ/OQ tools and FDA 21 CFR Part 11 software tools to help meet GMP requirements. Users may choose to have their IQ/OQ service performed by ForteBio qualified service personnel, or by third-party service personnel using ForteBio service products. The 21 CFR Part 11 software package provides electronic security, audit trails, user access management and other compliance features.

SUMMARY

Octet systems provide an automated solution for maximizing the efficiency of antibody detection and quantitation in biopharmaceutical development and manufacturing processes. A variety of assay types can be developed to suit various analytical requirements. The simple Dip and Read approach using the Octet platform enables high throughput, accurate and highly precise quantitation of monoclonal antibodies and other proteins at concentrations relevant to drug development, QC and manufacturing processes.

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PALL Life Sciences

Pall ForteBio Corp.
1360 Willow Road, Suite 201
Menlo Park, CA 94025
t: 888.OCTET-75
or 650.322.1360

Pall ForteBio Europe
5 Harbourgate Business Park
Southampton Road
Portsmouth, PO6 4BQ, UK
t: +44-(0)203 318 2840

Pall ForteBio Analytics (Shanghai) Co., Ltd.
917 Halley Road, Bldg 4
Zhangjiang High Tech Park
Shanghai, China 201203
t: +86-21-51320387

www.fortebio.com

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