SARTORIUS

Application Guide

August 11, 2020

Keywords or phrases:
Octet Bio-Layer Interferometry (BLI) system, CQA, crude sample, real-time kinetics, affinity, epitope screening, off-rate screening, hybridoma screening

Accurate Measurements on the Octet Platform Without Added Sample Purification Steps

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Abstract

Accurate quantitative assays performed on crude samples offer unprecedented time and cost savings. This overview highlights a few examples from drug discovery and process development where the Octet® system demonstrated a significant reduction in the analysis time over ELISA and HPLC by eliminating the need for purification, while still achieving high accuracy and precision. The total assay times were dramatically reduced with fewer assay steps and less labor time involved. The easy and versatile, fully integrated Octet assay has additional advantages over other label-free technologies like SPR, where sample washing steps are required.

Introduction

Bioanalytical systems have rapidly developed over the past 10 years for lead selection during cell line development. The advanced technologies for the timely and cost-effective screening of antibody or vaccine involve the quantitation, kinetics and other Critical Quality Attributes (CQA). One of the main challenges for traditional methods like ELISA and HPLC is the need for additional sample preparation steps to overcome matrix effects. The Octet platform is an established analytical technology that minimizes matrix effects when measuring, allowing for use with samples including antibodies, recombinant proteins, viruses and other macromolecules without the need for filtration or purification. Different types of crude samples have been successfully tested for real-time kinetics and affinity analysis, epitope screening and high-throughput titer during lead selection and bioprocess development (Table 1). A few selected case studies using crude samples for both lead selection and process development are discussed below.

A. Lead Selection and Optimization Using Crude Samples

1. High-Throughput Kinetic Screening of Hybridomas to Identify High-Affinity Antibodies Using the Octet System

Hybridoma technology remains the most common technique used to generate antibodies for therapeutic applications. Screening hybridoma supernatants for antigen-positive antibodies is a crucial step in screening and selection. Using the ELISA assay, clones that express high levels of a low-affinity antibody can give an equivalent signal to clones that express low levels of a high affinity antibody. As a consequence, superior clones can be overshadowed by inferior clones. A better assay method for selecting high-affinity antibodies would require screening hybridoma supernatants kinetically and would allow clones to be ranked and selected based on binding, but, more importantly, off-rate. The off-rate kinetic measurement is concentration independent and can be determined in samples in which the antibody concentration is unknown. Ranking and selecting antibodies based on off-rate would ensure that a high-affinity antibody expressed at low levels is not overshadowed or missed during primary screening.

The Octet RED384 instrument is a high-throughput, label-free platform, that can measure 16 samples in parallel by immersing ligand-coated biosensors into sample wells of a 96- or 384-well microplate. The fluidics-free configuration enables the Octet RED384 system to measure interaction kinetics in hybridoma supernatants in high throughput – more than 1000 samples a day. In a publication, Lad *et al.,* compared the Octet instrument to ELISA as a method to identify high-affinity antibodies by screening 2000 hybridoma supernatants. They found screening clones kinetically was an efficient method for identifying high-affinity antibodies. The results indicated that the highest-affinity antibody was identified by ranking the clones based on off-rate and would not have been identified using only the ELISA binding signal strength as the selection criterion[1].

2. High-Throughput Sialylation Measurement Using Lectins on an Octet Platform for Clone Screening

The detection and determination of sialic acid content is a critical quality control assessment in therapeutic drug development, as high sialylation levels on a glycoprotein have been shown to increase the stability of glycoproteins. Using the binding rate of *Maackia amurensis* lectin II (MALII) to (α-2,3)-linked sialic acids of proteins to detect and quantify sialic acid content was demonstrated by Kanvasri *et al.*[2]

Table 1: Examples of Crude Sample Screening by the Octet system.

Immobilized ligand	Analyte	Crude Sample	Assay	Biosensor	Ref
Anti-mouse (IgG) Fc	Fragment of the proprietary targets	Hybridoma supernatant	Kinetic/Off-rate screening	AMC	1
Fc-fusion proteins	MALII	Culture medium	Clone screening	Protein A	2
Cetuximab and Trastuzumab	ADA (Trastuzumab and Cetuximab)	Crude bacterial lysate	Anti-drug antibody off-rate screening	AR2G (Cetuximab), SA (b-Trastuzumab)	3
Protein L	Antibody fragment	*E. Coli* BL-21 (DE3)	Fab quantification	Protein L	4
Protein A and Protein G	Fc-fusion protein	Cell culture supernatant	Quantitation	Protein A and Protein G	5

A high-throughput method to quantify sialic acid in crude samples was developed by immobilizing a target protein onto a Protein A biosensor through the Fc region. They were able to screen 2000 clones within 24 hours and select clones containing high-affinity antibodies for further expansion and subsequent characterization. Using this method, they were able to identify several clones producing high-affinity antibodies that were missed by ELISA.

3. Off-Rate Screening for Selection of High-Affinity Anti-Drug Antibodies

Phage display of large recombinant antibody libraries enables rapidly increasing number of therapeutic antibodies, which requires corresponding detection reagents for monitoring the concentration of these drugs in patient samples and as positive controls for measurement of anti-drug antibodies. A typical bottleneck in antibody generation is ranking of the many candidates obtained after panning on the basis of antibody binding strength. Ideally, such method will work without prior labeling of antigens and with crude bacterial lysates. Ylera *et al.*, developed an off-rate screening method of crude *Escherichia coli* lysates containing monovalent Fab fragments obtained after phage display of the HuCAL PLATINUM® antibody library. They used the antibody drugs trastuzumab and cetuximab as antigen examples. Using the Octet RED384 instrument, they showed that antibody off rates could be reliably determined in crude bacterial lysates in a high throughput scale. They also demonstrated that the method can be applied to screening for high-affinity antibodies typically obtained after affinity maturation[3].

B. Process Development Using Crude Samples

1. The Octet Platform as an Alternative to HPLC for Measuring Product Concentration in Fermentation Broth

Escherichia coli (*E. coli*) is the most widely used expression system for the production of recombinant proteins because it offers high density fermentations and scale-up production at a minimum cost. However, one of the major challenges is the availability of an appropriate analytical method that can measure the concentration of the target analyte in complex matrices that fermentation broth usually offers. In addition to the complex of bioprocessing of protein therapeutics, antibody fragments concentration in the fermentation broth is quite low and there is a heterogeneous population of the product in the broth.

Commonly used methods for antibody fragment titer include ELISA, SDS-PAGE, western blotting and HPLC. However, ELISA has limited accuracy especially in the case of complex mixtures like culture broth and crude extracts. Also, this method cannot discriminate amongst the molecular variants of the product (for example, mass and charge isoforms) versus its native form. HPLC methods are well proven and widely accepted, similar to SDS-PAGE and western blotting, they all require laborious and time intensive sample pretreatment and analysis.

Using an Octet system, Rathore *et al.*, developed a biosensor-based screening for measuring the titer of a product[4]. An antigen-binding (Fab) fragment expressed in the periplasm of *E. coli* was accurately quantified by the Octet system. HPLC was also performed to show the correlation of the Octet instrument with HPLC. The screening workflow of sample analysis by the Octet system and HPLC is illustrated in Figure 1 and Table 2.

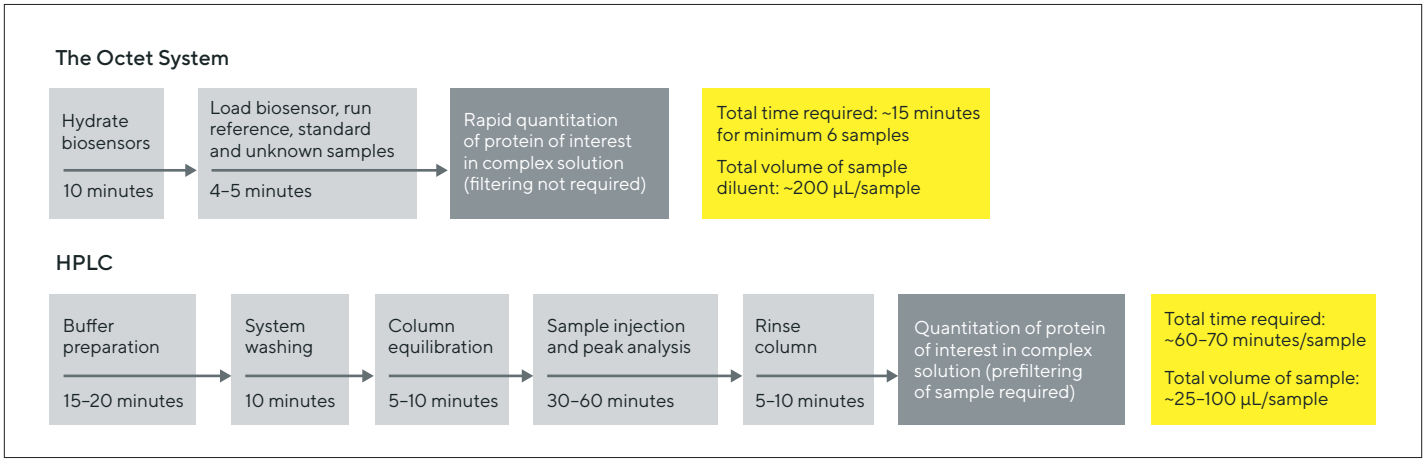

Figure 1: Illustration depicting the screening workflow in the Octet system and the HPLC system.

Table 2: Comparison of analysis attributes of ELISA, HPLC and the Octet System.

Method	ELISA	HPLC	Octet system
Number of assay steps	7	4	2
Labor time (hours)	3	0.5	< 0.2
Total time to results (hours)	> 6	10	0.5
Precision (% CV)	> 10	< 5	< 10

2. Rapid, reliable quantitation of Fc-Fusion protein in cell culture supernatants.

The cell line development group at Biogen IDEC needed a robust assay for the measurement of Fc-fusion protein in crude cell culture supernatants. The group had historically used HPLC for protein quantitation during screening and selection of promising mammalian clones at every scale-up step, from 96-well microplates to 3L-bioreactors. They wanted to replace the HPLC method due to its low throughput, cumbersome sample processing and long run times with a higher-throughput alternative that provided accurate, reliable results.

The Octet QK384 instrument was evaluated as an alternative to HPLC due to its many advantageous features and suitability for screening proteins in cell culture supernatant fluids. Octet systems have the ability to analyze crude samples, allowing users to bypass sample pre-processing. In addition, 96 samples could be analyzed in less than 30 minutes, expediting screening that took more than 19 hours to complete by HPLC. A higher throughput, automated screening workflow with significantly reduced analyst involvement was achieved via integration of the Octet system and a PerkinElmer (formerly Caliper Life Sciences) Sciclone robot[5].

Summary

We have only highlighted a few examples in this communication for drug discovery and process development using crude samples. As Anurag *et al.,* show in Table 1, significant reduction in the analysis time with high accuracy and precision have been achieved on Octet systems using crude samples when compared to ELISA and HPLC. The total assay time is dramatically reduced by less assay steps and labor time. Figure 1 has only demonstrated the screening workflow in both the Octet system and HPLC, however the easy and versatile use of Octet assays using crude samples also have many similar advantages over other label free technologies including ITC and SPR where sample washing step or purification may be required.

References

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