*forté*BIO®

*A Division of **Pall Life Sciences***

PALL® Life Sciences

Converting an ELISA Assay into an Octet Quantitation Assay

Sae Choo, Director, Assay Development, schoo@fortebio.com

INTRODUCTION

Quantitation assays on the Octet platform have many similarities to enzyme-linked immunosorbent assays (ELISA). Both are performed on a solid support on which the capture molecule is immobilized and the analyte is bound from solution. Signal reported in the assay is either directly or inversely proportional to the amount of bound analyte. In fact, Octet quantitation assays can be considered automated forms of ELISA. The conversion to an Octet assay often involves simply re-optimizing and/or validating the conditions and configurations of the already existing ELISA assay. However, in some cases where the minimum essential requirements are stringent, more development work is required.

It is often beneficial to convert ELISA assays to the Octet platform as it allows the scientist to:

1   Choose from a number of assay formats (label-free direct binding, sandwich, sandwich followed by signal amplification, etc.) to suit detection limit requirements

2   Detect low-affinity analytes often missed by ELISA

3   Minimize handling via automated and wash-free steps

4   Fully recover and re-use samples and reagents

5   Regenerate the assay surface and re-use for some binding pairs (*e.g.*, Protein A/human IgG).

This article provides guidance on converting an existing ELISA-based assay to an Octet assay. The process can be broken down into five steps:

1   Defining assay requirements

2   Selecting a biosensor type

3   Selecting assay format

4   Minimize non-specific binding (NSB)

5   Optimizing assay buffer

Details on each of these steps are presented in the following sections.

DEFINING ASSAY REQUIREMENTS

Conversion activity can start with defining the minimum assay requirements. Sensitivity and throughput requirements often become critical factors in determining the Octet instrument, biosensor type and assay format, while the sample matrix has the biggest impact on reagent formulation and the blocking protocol. Sample volume requirements also influence the choice of instrument (Octet QK, QKe and RED require 80–200 µL while Octet QK384 and RED384 require 40–80 µL). Sample volume also indirectly influences the sensitivity of detection when dilution is required to compensate for limited availability of sample. Physical characteristics (pI, size, polymeric status, hydrophobicity, stability, etc.) of the analyte should be considered when selecting the surface and the immobilization protocol.

SELECTING A BIOSENSOR TYPE

There are four types of biosensors to be considered in building quantitation assays on the Octet platform. They are Streptavidin (SA), Aminopropylsilane (APS), Amine-Reactive (AR) and analyte-specific biosensors.

SA, APS, and AR biosensors can be used to build custom quantitation assays for analytes that are process-specific.

SA biosensors are most often preferred for their flexibility to accommodate a variety of capture molecules (ligand) through streptavidin/biotin interaction and its superior surface capacity

Assay Features	1-Step	2-Step	3-Step
Pictorial Representation			
Assay Steps	1. Bind analyte	1. Bind analyte 2. Bind secondary reagent	1. Bind analyte – secondary antibody complex 2. Bind HRP-loaded antibody 3. Incubate in precipitating substrate for HRP
Typical Assay Time	30 min (Octet QK, QKe, RED96) 15 min (Octet RED384, QK384)	1 hr 30 min (Octet QK, QKe, RED96) 1 hr 15 min (Octet RED384, QK384)	2 hr (Octet QK, QKe, RED96) 1 hr 30 min (Octet RED384, QK384)
Typical Concentration Range	Low mg/mL to low ng/mL	Low ng/mL to low pg/mL	Low ng/mL to low pg/mL
Advantages	Single incubation step — fast, easy, reduces reagent expenses Low affinity analytes detected — even those missed by ELISA NO labeled reagents Kinetic parameters can be measured Allows regeneration and re-use of biosensor in most cases	Two incubation steps — still fast, easy, reduces reagent expenses in comparison to ELISA Higher sensitivity of detection, down to low pg/mL, depending on assay NO labeled reagents Automated and no-wash assay minimizes handling	Similar to most ELISA assays in format — but faster and easier Excellent sensitivity — down to low pg/mL, depending on assay Automated and no-wash assay minimizes handling

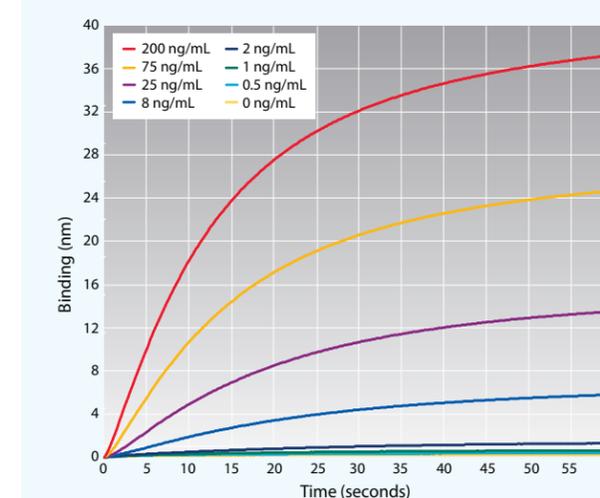TABLE 1: Octet assay formats and features.

FIGURE 1: Raw data from the final amplification step of the CHO HCP assay using Octet RED96 system.
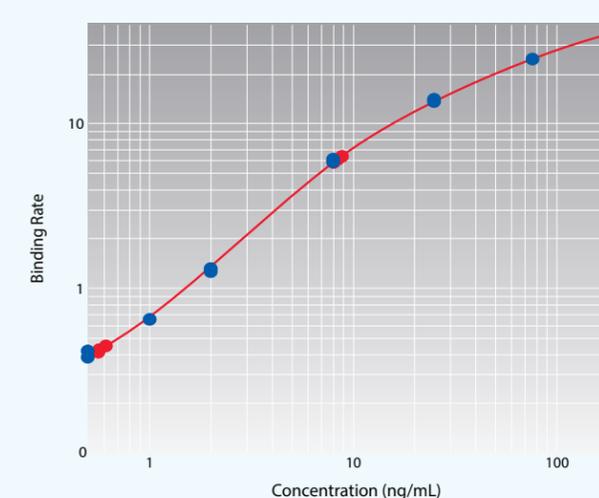
FIGURE 2: Standard curve showing standards (n = 3 for each standard) and unknown samples (n = 8 for each unknown). The lower limit of detection in this assay was less than 0.5 ng/mL.

Spike Conc. (ng/mL)*	Calculated Conc.	%CV of Calculated Conc.	% Recovery
200	201.6	4	101
8	8.5	4	106
0.5	0.57	7	114

* CHO Cell Protein, n = 8 for each.

TABLE 2: Accuracy and robustness of CHO host cell protein quantitation. In this assay, CVs of less than 10% were achievable across the entire dynamic range.

derived from the use of specially designed cross-linked streptavidin conjugates. There are several advantages of immobilizing the ligand that is labeled with a long-chain biotin at a low molar coupling ratio (biotin:ligand):

1 Loss of binding capacity due to cross-linking and steric hindrance are reduced.

2 Biosensors can be prepared in batch mode and stored for later use.

3 The biotin-ligand solution may be used to prepare multiple batches of biosensors.

4 The strong, nearly irreversible binding between the biotinylated ligand and the Streptavidin Biosensor allows easy regeneration of the ligand-loaded biosensor.

APS biosensors are ideal for immobilizing ligands that are not suitable for covalent linking chemistry. The mode of immobilization is a combination of hydrophobic and/or electrostatic interactions. The APS Biosensor surface is somewhat more hydrophobic in comparison to the surface of ELISA microtiter plates , so the stability of the ligand on the biosensor surface should be tested prior to loading to avoid denaturation.

AR biosensors can be used to covalently immobilize amine group containing ligands onto carboxylate groups on the biosensors. Batch mode preparation and regeneration of the biosensor are possible.

Analyte-specific biosensors are pre-immobilized with specific capture proteins such as anti-human IgG Fc, anti-murine IgG (Fab')2, Protein A, Protein G, and anti-penta HIS. These ready-to-use biosensors are suited for quantitative analysis of human IgG, mouse IgG, rat IgG, all proteins that bind Protein A, those that bind Protein G, and penta-HIS-tagged proteins, respectively.

SELECTING ASSAY FORMAT

The choice of an assay format is dependent on the concentration range of analyte to be quantified. Octet systems offer the advantage of a direct binding assay (also called 1-step), that generally affords a dynamic range of detection from low ng/mL to low mg/mL, depending on the analyte. The direct binding assay is fast, easy and eliminates the need for secondary reagents and steps. It also allows regeneration of the biosensor in some cases.

Multi-step assays provide enhanced sensitivity down to low pg/mL, depending on the analyte. Octet systems measure signal as a function of the thickness and density of the analyte binding layer, so increasing analyte binding translates to bigger signals. When analyte is present in low concentrations and the binding signal is low even after a long incubation, building additional layers of secondary reagents over the analyte binding layer enhances signal.

Such multi-layer assays include sandwich-style assays (also called 2-step assays), which use the biosensor to capture analyte in the first step, followed by use of a second antibody to sandwich the analyte in the second step. For even more signal amplification, an enzyme-linked sandwich assay (also called a 3-step assay) captures analyte bound by two separately-labeled capture molecules to the biosensor in the first step, binds an HRP-conjugated antibody to the complex in the second step, and precipitates a substrate directly onto the biosensor surface in the third step.

MINIMIZE NON-SPECIFIC BINDING (NSB)

Managing NSB and matrix effects are critical parts of the assay development process that ensure acceptable specificity and sensitivity. The major sources of NSB are hydrophobic, electrostatic, and cross-reactive interactions between the molecules on the biosensor surface and in the solution. In addition to NSB, unrelated proteins and other components of the sample matrix often cause assay interference.

The most effective way to minimize such effects is passivation of the biosensor surface by including blocking step(s) prior to sample incubation. Often, blocking buffer formulation needs to be matched to the sample matrix (e.g., block with the serum for immunogenicity sample in serum).

OPTIMIZING ASSAY BUFFER

Assay buffer formulation has a significant effect on NSB, sample matrix interferences and signal over background. The goal of optimizing reagent formulation is to maximize specific signal and minimize NSB. A direct adaptation of previously developed ELISA formulation may be sufficient in most cases, unless the formulation contains components or conditions that are not compatible with ForteBio biosensors. Examples of such incompatible reagents include those with low pH (<4), high pH (>10), and certain types of organic solvents. The characteristics of the analyte (pI, hydrophobicity and stability) and sample matrix components are important to keep in mind when developing an assay buffer.

On a related note, the rinsing step employed in Octet assays is often performed in physiological buffers such as TBS and PBS in the presence of a detergent.

The following is a list of common assay buffer components that are useful to keep in mind during optimization.

1   Salt: high salt concentrations slow down the reaction, reduce stickiness of antigens with very high/low pI values, reduce charge-induced NSB and denature proteins in solution when needed (*e.g.*, Protein A contamination assay sample pre-treatment buffer).

2   Buffer capacity: higher buffer capacity minimizes pH changes and stabilizes sample solutions.

3   Non-specific antibodies: reduce NSB from multi-species cross-reactions.

4   Detergents: reduce sample aggregation, reduce hydrophobic NSB and reduce sample coating out of very low concentration samples.

5   Bulk proteins (BSA, casein, antibodies): serve as blocking agents, reduce sample coating out of solution, and stabilize analyte proteins in solution in other ways.

6   Sugars (trehalose, dextran, sucrose): stabilize some proteins, enhance signal by increasing effective concentration in solution.

7   PEG: helps reduce non-specific binding and can enhance signal in some cases by increasing the effective concentration of proteins in solution.

8   pH: low pH may often reduce the affinity of competing interactions and improves the proportion of ligand-analyte binding to the biosensor.

9   Dilution: to minimize matrix effects.

CONCLUSIONS

ELISA-based assays and Octet quantitation assays share many similarities. Therefore, conversion of a pre-configured ELISA assay to the Octet platform may only require transfer of assay conditions. When assay conditions need re-optimization on the Octet platform, considerations are often similar to those employed in ELISA.

The Octet platform's direct binding assay method is simple, fast and accurate; the multi-step methods offer high sensitivity and expanded dynamic range. The automated assay formats enhance time-to-results and walk-away time and reduce operating expenses.