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Converting an ELISA Assay Into an Octet® Quantitation Assay

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Abstract

Enzyme-linked immunosorbent assays (ELISA) are routinely used to quantitate molecules and despite their popularity, these assays are labor- and time-intensive. Quantitation assays on the Octet® platform can be considered automated forms of ELISA but allow a myriad of benefits compared to standard ELISA assays, notably the ability to detect lower affinity interactions and a reduced hands-on time for the scientist. As with ELISA, the signal reported in quantiation assays is either directly or inversely proportional to the amount of bound analyte.

The conversion of many existing ELISA to an Octet® assay typically requires simple re-optimization and validation of the conditions and configurations. This application note describes the key steps for ensuring successful conversion of an ELISA-based assay to the Octet®, including defining the assay requirements, selecting the biosensor type and assay format, minimizing non-specific binding, and optimizing the assay buffer.

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® QKe, RED, and R8 require 80–200 µL while Octet® RH16 and RH96 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.

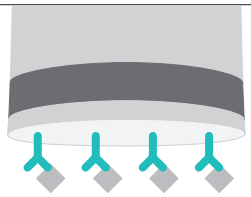
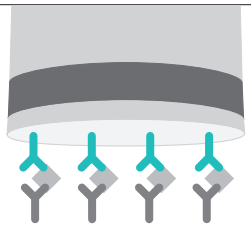
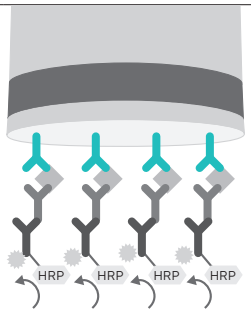
SA Biosensors are most often preferred for their flexibility to accommodate a variety of capture molecules (ligand) through streptavidin/biotin interaction and its superior surface capacity derived from the use of specially designed cross-linked streptavidin conjugates. There are several advantages of 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.

Table 1: Octet® assay formats and features.

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® QKe, R8) ▪ 15 min (Octet® RH16, RH96)	▪ 1 hr 30 min (Octet® QKe, R8) ▪ 1 hr 15 min (Octet® RH16, RH96)	▪ 2 hr (Octet® QKe, R8) ▪ 1 hr 30 min (Octet® RH16, RH96)
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

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.

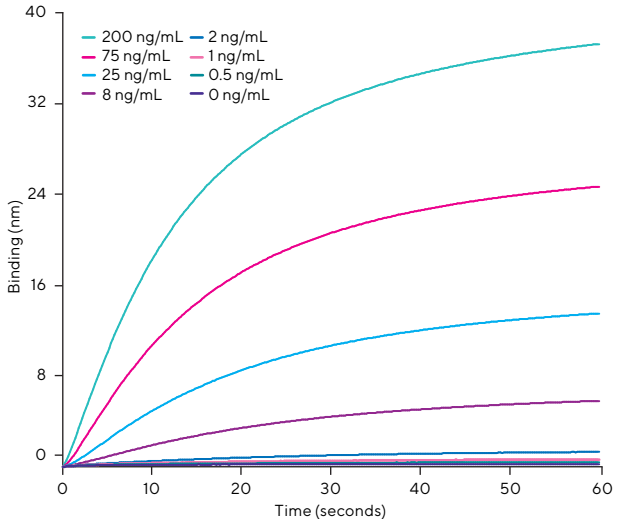


Figure 1: Raw data from the final amplification step of the CHO HCP assay using Octet® R8 system.

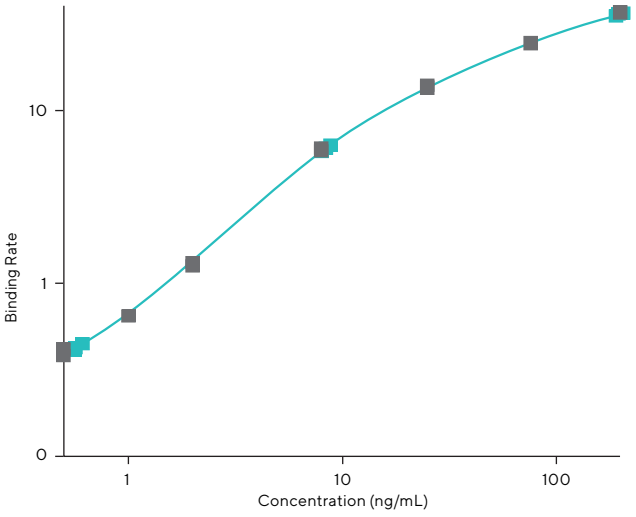


Figure 2: Standard curve showing standards (n = 3 for each standard) and unknown samples (n = 8 for each unknown). The lower limit of detection in this assay was less than 0.5 ng/mL.

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

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 eac

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 Sartorius 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.

Conclusion

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.

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