Octet® BLI Quantitation Assays: Method Development Guideline

Technical Note

Scope
This Technical Note provides insight into the procedure for developing and qualifying biological ligand binding quantitation assays on Octet® R series instruments.

Executive Summary

Design and development of biological assays that conform to USP <1032> requires parameters for accuracy, precision, linearity and limit of quantitation (LOQ) to be established. This document is intended as a general guideline for developing product-specific ligand binding quantitation assays on Octet® R series instruments.

Careful design, development and qualification of biological assays allows the user to subsequently evaluate CQA in factors such as a change in production process, reference material or critical reagents. Therefore, the time, effort and resources used in developing a USP <1032> compliant assay can yield many benefits in its future use.

1. Materials Required

- Sartorius Biosensors: Select the biosensor surface chemistry that is appropriate for your analyte of interest and sample matrix
- Black 96- or 384-well plates: Compatible sample plates include Greiner Bio-One black 96-well polypropylene, flat-bottom plates (Part No. 655209), Greiner Bio-One black 384-well polypropylene, flat-bottom plates (Part No. 781209), or the Sartorius tilted-well 384 well, polypropylene plates (Part No. 18-5080)
- Regeneration buffer: Refer to the technical note for the selected biosensor for the appropriate regeneration buffers and conditions for the biosensor of choice
- Octet® Sample Diluent: Sartorius Part No. 18-1104
- Standards: Same molecule as the sample

2. Assay Format

2.1 Direct quantitation

The concentration of an analyte can be measured directly in a one-step quantitation assay using Octet® BLI systems. Measurement of product concentration such as mAbs, recombinant proteins, virus and virus-like particles (VLPs) etc., can be achieved in a one-step Dip and Read assay in a direct quantitation assay format. Off-the-shelf biosensors such as Protein A (ProA), Protein G (ProG), Protein L (ProL), Anti-Human IgG (AHQ and AHC2) or Anti-Murine IgG (AMQ) Biosensors can be used for the quantitation of human and mouse mAb and for Fc fusion proteins. Streptavidin-based biosensors can also be customized using specific biotinylated antibodies to quantify recombinant proteins, viruses and VLPs.

2.1 Indirect quantitation

Indirect quantitation assay formats (two- or three-step assays) are used when the analyte in the samples exists in low concentrations and requires higher assay sensitivity for accurate quantitation. These analytes include contaminants such as residual protein A (RPA) and host cell proteins (HCPs) in biological products and anti-drug antibodies (ADAs) in subject serum.

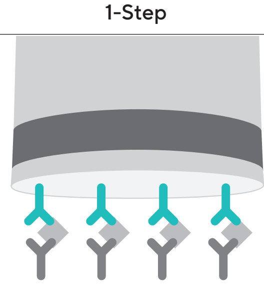
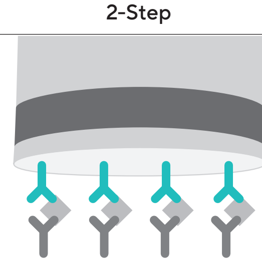
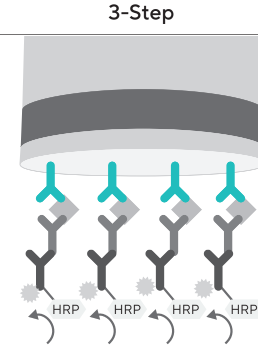
Assay features	1-Step	2-Step	3-Step
Pictorial representation			
Assay steps	1 Bind analyte	1 Bind analyte 2 Bind secondary reagent	1 Bind analyte – secondary antibody complex 2 Bind HRP-loaded antibody 3 Incubate in precipitating substrate for HRP
Typical assay time for 96 samples	• 30 min (Octet® R8 system) • 15 min (Octet® RH16 system) • 2 min (Octet® RH96 system)	• 1 hr 30 min (Octet® R8 system) • 1 hr 15 min (Octet® RH16 system) • 1 hr (Octet® RH96 system)	• 2 hr (Octet® R8 system) • 1 hr 30 min (Octet® RH16 system) • 1 hr (Octet® RH96 system)
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

Figure 1: Octet® BLI quantitation assay types.

Table 1: Biosensor and assay formats for Octet® quantitation assays.

Biosensor/Kit	Surface chemistry	Analyte	Assay format	Regeneration
ProA	Recombinant Protein A	Various IgG species	Direct; One-step	Yes
ProG	Recombinant Protein G	Various IgG species	Direct; One-step	Yes
ProL	Recombinant Protein L	Various IgG species	Direct; One-step	Yes
AMQ	Anti-murine IgG Fv	Mouse IgG	Direct; One-step	No
AHC2	Anti-human IgG Fc	Human IgG	Direct; One-step	Yes
AHQ	Anti-human IgG Fc	Human IgG	Direct; One-step	No
FAB2G	Anti-human Fab CH1	Human Fab and F(ab')2	Direct; One-step	Yes
HIS1K	Anti-penta-HIS (Qiagen)	HIS-tagged protein	Direct; One-step	Analyte-dependent
HIS2	Anti-penta-HIS (MBS)	HIS-tagged protein	Direct; One-step	Analyte-dependent
GST	Anti-GST	GST-tagged protein	Direct; One-step	Yes
SAX	High Precision Streptavidin	Binding partner to immobilized ligand	Direct; One-step customized	Analyte and ligand dependent
SAX2*	High Precision Streptavidin 2.0	Binding partner to immobilized ligand	Direct; One-step customized	Analyte and ligand dependent
Anti-CHO HCP Kit	Anti-CHO Host Cell Protein	CHO HCP	Indirect; Three-step	No
RPA Kit	Anti-protein A	Residual protein A	Indirect; Two-step	No

* SAX2 Biosensors are suitable for drug discovery and regulated environments providing minimal lot-to-lot biosensor loading variance.

3. Biosensor Selection

Depending on the analyte to be measured and the expected concentration of the analyte, the user will have to choose the appropriate biosensor and assay format (see Table 1).

4. Biosensor Hydration

Select the appropriate biosensor surface. Pipette 200 µL of assay buffer into the appropriate 96-well microplate. Pipette buffer only in wells corresponding to the number of biosensors intended for immediate use. Hydrate the biosensors passively on the lab bench for at least 20 minutes.

Note: Biosensors should be hydrated in a matrix identical to the sample matrix. For example, if your analyte is in growth media, the same media type should be used to hydrate the biosensors.

5. Development of the Calibration (Standard) Curve

Quantitation assays on Octet® BLI systems require the use of a reference or standard curve obtained from a molecule identical to the test molecule with pre-determined concentrations. If a purified molecule stored in buffer is used for the standard curve development, the relevant sample concentrations should be spiked into the desired assay matrix. It is recommended that the stock sample be prepared in the assay matrix first and be used for the preparation of the concentration points. As standard curves may be nonlinear inherently, more concentration points are required to define the fit over the standard curve range. As such, a minimum of six duplicate non-zero calibrator concentrations are required to construct the calibration curve covering the entire range including the lower limit of quantitation (LLOQ).
The concentration-response relationship is most often fitted to a 4- or 5-parameter logistic model, although other models may be used with suitable validation. To achieve optimal accuracy, it is critical that the calibrator samples are prepared in the same matrix as the test samples.

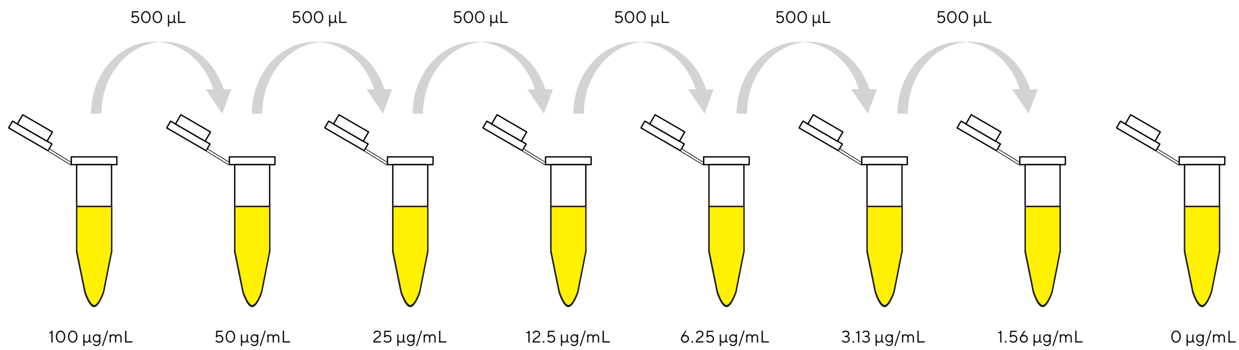


Figure 2: Two-fold serial dilution of the stock reference material.

As an example, the full dynamic range of an IgG direct quantitation assay on the Octet® BLI platform using ProA Biosensors is 0.025–2000 µg/mL. However, actual assay dynamic range is dependent on sample type, sample contact time and shaking speed. For samples in the lower concentration range (0.025–100 µg/mL), a sample contact time of 5 minutes and a shaking speed of up to 1000 RPM is required. For samples at the mid-concentration range (1–700 µg/mL), a sample contact time of 2 minutes and a shaking speed of 400 RPM may be sufficient. For samples at the higher concentration range (200–2000 µg/mL), a sample contact time of 2 minutes and a shaking speed of 200 RPM may be optimal. It is recommended that a control analyte sample with a known concentration but not part of the standard curve be used to verify the optimal conditions.

6. Sample Preparation

6.1 When starting with 100 µg/mL for example, pipette 500 µL of sample diluent into eight empty microfuge tubes and perform a two-fold serial dilution of the stock reference material to in sample diluent to obtain a set of concentration between 1.56 and 100 µg/mL as shown in Figure 2.

7. Analyte Quantitation

To ensure minimal matrix interference, it is recommended to dilute the samples with sample diluent by a dilution factor of at least 1:10.

7.1 Add 1 part of sample to 9 parts of sample diluent in a microfuge tube.

7.2 Agitate well using a benchtop vortex mixer.

7.3 Pipette 200 µL of the standards, samples and buffers into a black 96-well microplate in triplicate according to the plate map shown in Figure 3.

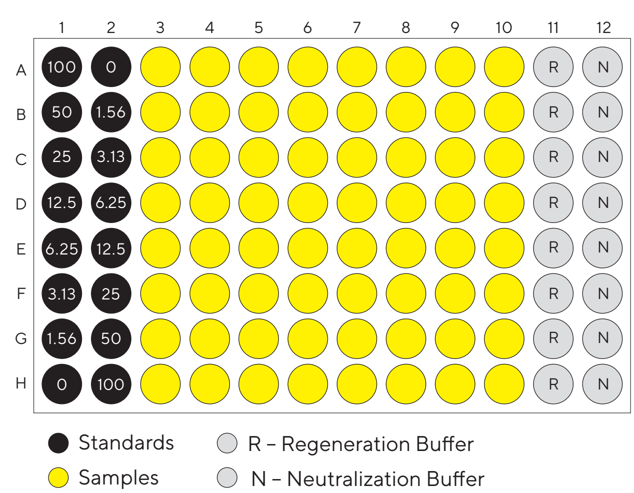


Figure 3: Sample plate layout for analyte quantitation.

8. Data Analysis

8.1 In Octet® BLI Analysis Software, locate the directory where the data was saved, double-click to load the assay data and select the loaded data to open it.

8.2 Proceed to the Results tab, select the appropriate Standard Curve Equation and click Calculate Binding Rate to process the data.

8.3 The standard curve will be presented and the standard/sample binding rate, calculated concentrations, related standard deviation (SD) and coefficient of variation (CV) will be presented in the results table.

9. Specificity

It is important to assess specificity and show that the assay unequivocally assess the analyte in the presence of other components that are in the matrix. To ensure that the host cell proteins in cell culture supernatants do not bind non-specifically with the biosensors, conditioned media obtained by culturing host cells that do not express the product is required. No binding signal should be observed when the biosensors are introduced to the conditioned media.

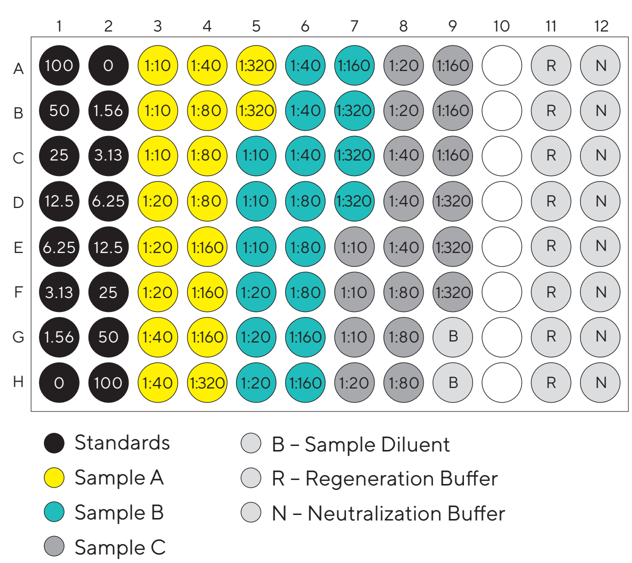


Figure 4: Sample plate layout for dilution linearity testing.

10. Dilution Linearity

To demonstrate that a sample with a concentration higher than the upper limit of quantification (ULOQ) can be diluted to a concentration within the assay working range and still give a reliable result, a dilution linearity test will need to be performed.

10.1 Dilute a sample which has a concentration above the ULOQ over a wide range of dilution factors (e.g. 1:10, 1:20, 1:40, 1:80, etc.) with the sample diluent to get the sample concentration within the assay standard curve range.

10.2 Prepare a sample plate with standards and samples diluted at different dilution factors in duplicate as shown in Figure 4.

10.3 Set up a basic quantitation assay with regeneration using Octet® BLI Discovery Software using the assay settings in section 8.1 and run the assay.

10.4 Load the data into Octet® BLI Analysis Software for data analysis and tabulate the results in the format shown in Table 2.

Table 2: Dilution linearity results.

Sample ID	Avg. well conc. (µg/mL)	Dilution factor	Calc. conc. (µg/mL)	CV (%)	Avg. sample conc. (µg/mL)
A		1:10			
		1:20			
		1:40			
		1:80			
		1:160			
		1:320			
B		1:10			
		1:20			
		1:40			
		1:80			
		1:160			
		1:320			
C		1:10			
		1:20			
		1:40			
		1:80			
		1:160			
		1:320			

11. Accuracy

The accuracy of the assay reflects the closeness of the value with the perceived true value. Typically, a reference material of known concentration is required to assess the accuracy of the assay.

12. Repeatability

12.1 Intra-plate precision

When assessing quantitation assay repeatability on the Octet® BLI platform, it is important to determine the inter-channel variation and the inter-cycle variation (regeneration efficiency).

12.1.1 Prepare a sample plate following the plate map as shown in Figure 5.

12.1.2 Set up a basic quantitation assay with regeneration using Octet® BLI Discovery Software usting the assay settings in section 8.1 and run the assay.

12.1.3 Load the data into Octet® BLI Analysis Software for data analysis and calculate the intra-plate average concentration and % CV and % recovery of the samples as shown in Table 3.

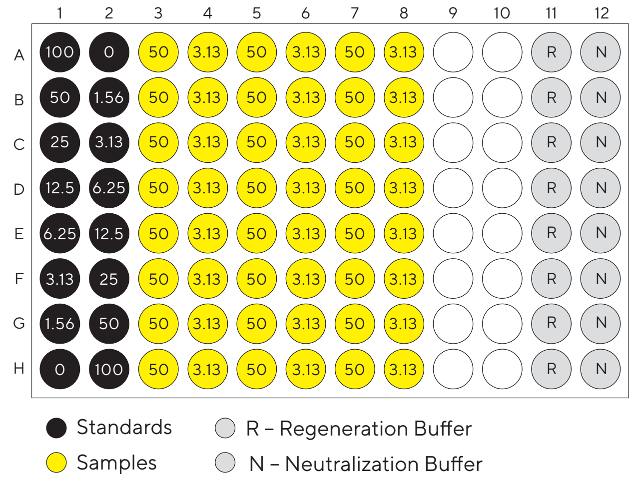


Figure 5: Sample plate layout for intra-plate precision testing.

12.2 Inter-plate precision

12.2.1 To determine inter-plate precision, perform a triplicate of the assay in Section 12.1 to determine the variation (CV) between plates.

12.2.2 Using Octet® BLI Analysis Software, determine the intra-plate average concentration of the 50 µg/mL and 3.13 µg/mL samples.

12.2.3 Calculate and tabulate the inter-plate average concentration of the 50 µg/mL and 3.13 µg/mL samples, % CV and % recovery as shown in Table 4.

Table 3: Intra-plate precision results.

Sample (µg/mL)	Intra-plate avg. conc. (µg/mL)	CV (%)	Recovery (%)
50			
3.13			

Table 4: Inter-plate precision results.

Sample (µg/mL)	Plate	Intra-plate avg. conc. (µg/mL)	Inter-plate avg. conc. (µg/mL)	CV (%)	Recovery (%)
50	1				
	2				
	3				
3.13	1				
	2				
	3				

13. Intermediate Precision

The intermediate precision assesses the impact that within-lab variations such as different days, different analysts or different equipment has on the repeatability of the assay.

13.1 Day-to-day Precision. Repeat the assay in section 12 while varying days and record results in Table 5.

13.2 Analyst-to-analyst Precision. Repeat the assays in section 12 while varying analysts and record results in Table 6.

Table 5: Day-to-day precision results.

Sample (µg/mL)	Day	Intra-plate avg. conc. (µg/mL)	Inter-plate avg. conc. (µg/mL)	CV (%)	Recovery (%)
50	1				
	2				
	3				
3.13	1				
	2				
	3				

Table 6: Analyst-to-analyst precision results.

Sample (µg/mL)	Analyst	Intra-plate avg. conc. (µg/mL)	Inter-plate avg conc. (µg/mL)	CV (%)	Recovery (%)
50	1				
	2				
	3				
3.13	1				
	2				
	3				

Reference

USP guidelines chapters 111, 1032, 1033, 1034.

Disclaimer

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