SARTORIUS

Octet® ProA-MAX Biosensors

For Quantitation of High-Titer Human IgG

Technical Note

Scope: This Technical Note details the use of Octet® ProA-MAX Biosensors for the quantification of high-titer hIgG samples.

Keywords or phrases: Octet®, Bio-Layer Interferometry, BLI, hIgG Titer Quantification, Human IgG, Human IgG1, Polyclonal IgG, high titer, Octet® ProA Biosensors, Octet® ProA-MAX, ProA-MAX, Bioprocessing, Cell Line Development, CLD, IgG titer

Abstract

Human IgG antibodies are widely used in therapeutic development and often serve as critical drug candidates. Advances in recombinant protein technology and cell line development have improved production efficiency, resulting in higher antibody expression levels and an increasing need for technologies that can efficiently quantify high-titer IgG samples. Octet® ProA-MAX Biosensors enable rapid, accurate, high-throughput quantification of human IgG titers up to 12000 µg/mL in both crude and purified samples, without the need for sample purification or serial dilution. As a result, they simplify workflows and reduce time to results, providing an excellent solution for high-titer IgG quantification at various stages of bioprocessing.

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Introduction

The Octet® Bio-Layer Interferometry (BLI) platform, in combination with Octet® Protein A (ProA) Biosensors, has been widely used for IgG titer quantification during Cell Line Development (CLD), as it offers rapid and accurate high-throughput analysis of both crude and purified samples. However, broader use of the Octet® ProA Biosensors in downstream bioprocessing has been limited by their dynamic range of 0.025–2000 µg/mL, which requires serial dilution of samples with higher-titer samples and thereby increases the complexity and time required for analysis.

To address this limitation, Sartorius developed the Octet® ProA-MAX Biosensor, the next generation of Octet® ProA Biosensors, designed to significantly increase the upper detection limit while preserving the high accuracy and precision. This expanded dynamic range enables direct analysis of IgG samples with titers from 100 µg/mL to 12000 µg/mL without serial dilution (Table 1). Similar to the existing Octet® ProA Biosensors, Octet® ProA-MAX Biosensors can be regenerated and reused up to 10 times, providing the same versatility and cost-effectiveness for IgG quantification.

	Octet® ProA Biosensor	Octet® ProA-MAX Biosensor
Linear Range, µg/mL	1–300	100–5000
Dynamic Range, µg/mL	0.025–2000	100–12000

Table 1. Comparison between the Octet® ProA and Octet® ProA-MAX Biosensors. The linear range is the concentration range in which the binding rate and hIgG concentration have a correlation coefficient R^2>0.99 when calculated using a linear fitting model.

Specificity

The Octet® ProA-MAX Biosensor are pre-immobilized with a custom-engineered Protein A-like ligand that demonstrates high specificity to hIgG1, polyclonal human IgGs, and the human Fc region. It shows much lower affinity and specificity to hIgG2, IgG3 and IgG4 subclasses, as well as to human Fab fragments (Figure 1). Furthermore, the Octet® ProA-MAX Biosensors show strong binding to monkey, dog, cat and rabbit IgGs, but weak or no binding to mouse and rat IgGs. Thus, the Octet® ProA-MAX Biosensors are extremely useful for quantification of a wide range of IgGs at different stages of development and bioprocessing, including later stages of cell line development, process development, and QC in both crude and purified samples.

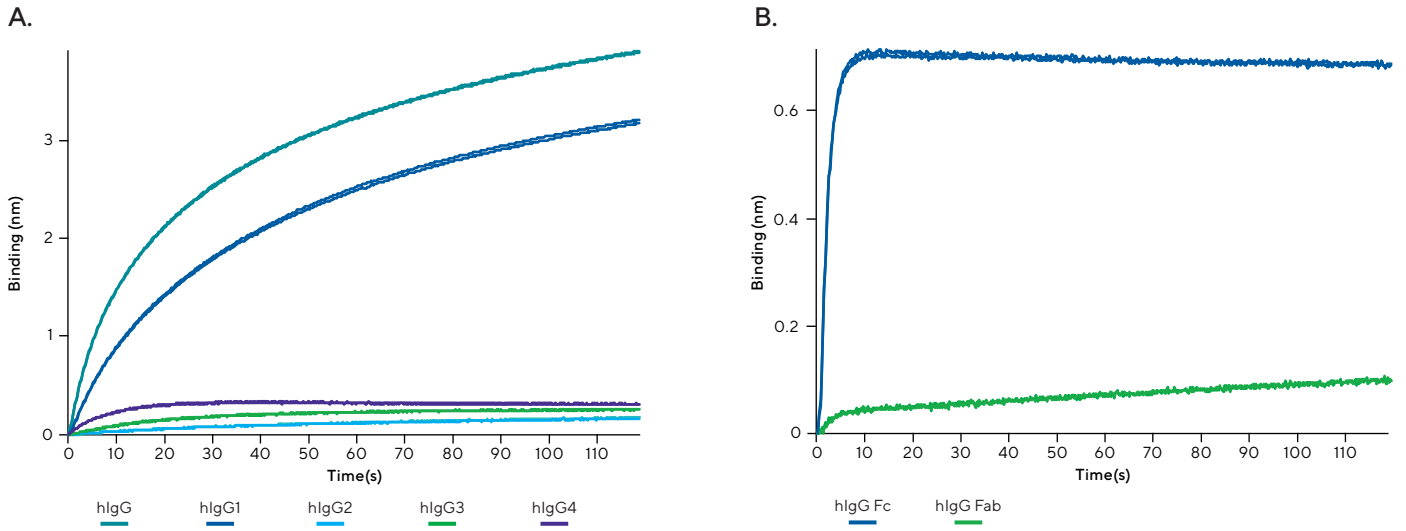

Figure 1. Specificity of the Octet® ProA-MAX Biosensor. Stronger binding to hIgG1, polyclonal IgG and human Fc and weaker or no binding to hIgG2, hIgG3, hIgG4, and human Fab. All samples were tested at concentrations of 1000 µg/mL.

Quantitation Assay Workflow

The Octet® ProA-MAX Biosensors can be used to quantitate both crude and purified IgG1 and polyclonal IgGs in the range of 100–12000 µg/mL without the need for serial dilution. The quantitation workflow using ProA-MAX Biosensors is the same as for existing ProA Biosensors and is outlined in Figure 2. It begins with the creation of a standard calibration curve by analyzing calibration standards with known titers, followed by analysis of the unknown samples and determination of their concentrations from the calibration curve. As with any Octet® quantitation assay, the calibration standards should closely represent the test samples, including the sample matrix or media, to ensure accurate analysis. A representative example for polyclonal IgG quantitation is shown in Figure 3 and Table 2.

Known Titer, µg/mL	Average Calculated Titer, µg/mL (n=3)	%CV (n=3)	%Recovery
12000	12100	4.4%	100.7%
10000	10000	2.5%	100.1%
8000	7915	3.4%	98.9%
5000	5038	2.5%	100.8%
2000	2001	1.1%	100.1%
1000	1005	3.2%	100.5%
500	497	2.2%	99.5%
200	196	1.8%	98.4%
100	100	7.0%	100.0%

Table 2. Calculated titer, %CV and %Recovery for hIgG quantitation assay shown in Figure 3.

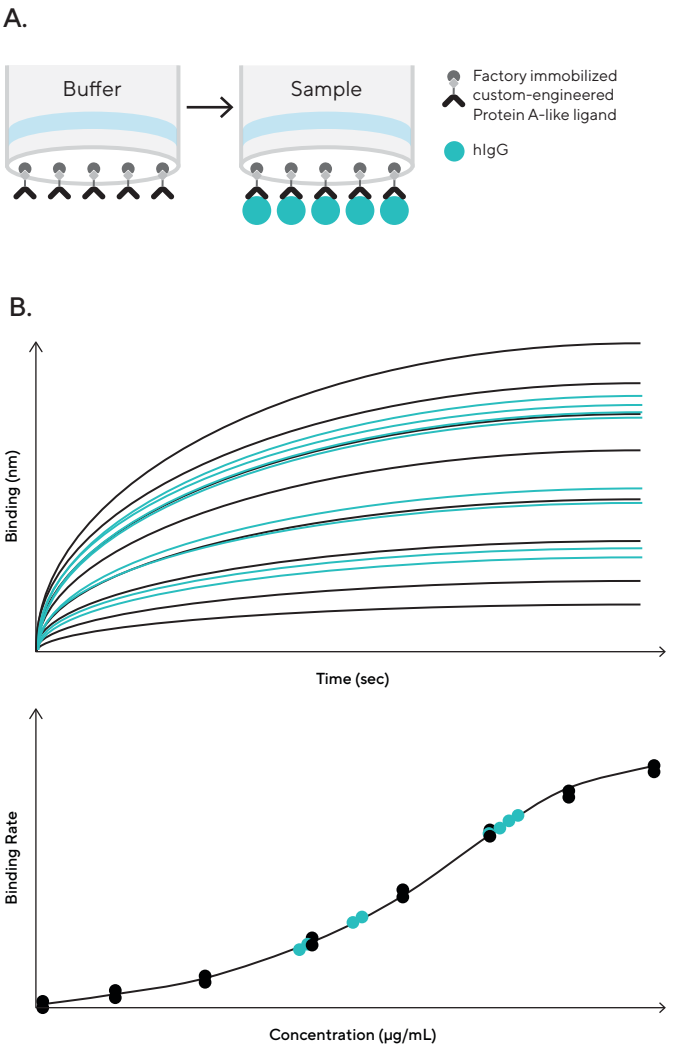

Figure 2. hIgG Quantitation Assay Workflow Using the Octet® ProA-MAX Biosensors. (A) Quantitation assay workflow starts with equilibration of the Octet® ProA-MAX Biosensors in the assay buffer (Buffer) followed by quantitation of hIgG (Sample). (B) Schematic representation of quantitation analysis of hIgG standards and unknown samples on the Octet® BLI platform.

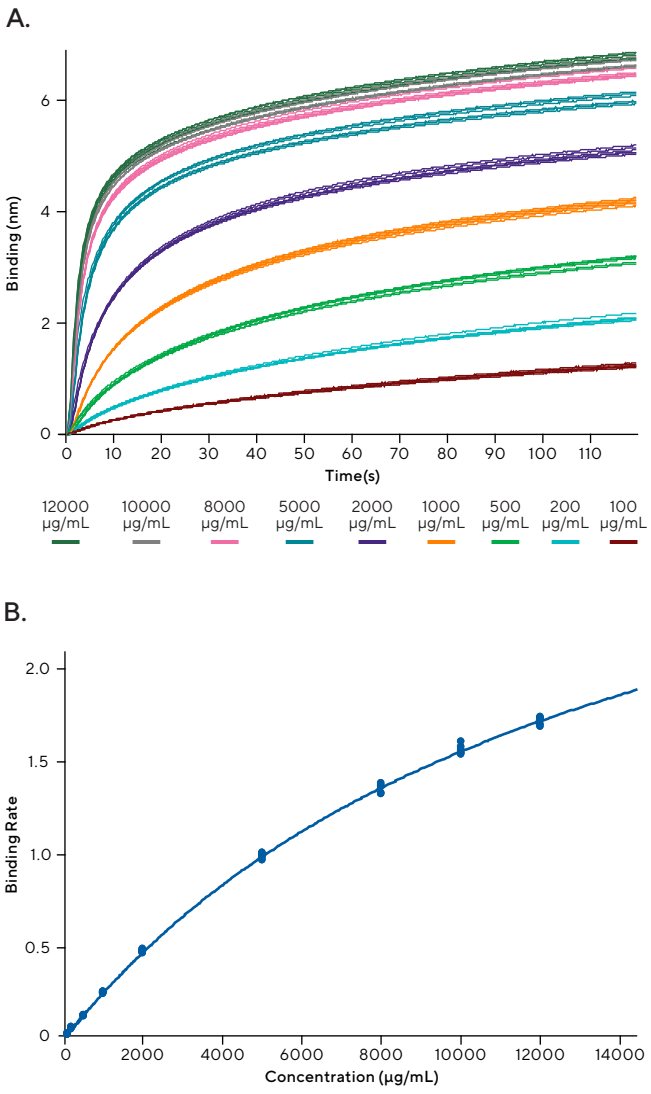

Figure 3. Quantitation of purified hIgG sample in Octet® Sample Diluent buffer using the Octet® ProA-MAX Biosensors. (A) hIgG dose-response for concentrations within the dynamic range of 100–12000 µg/mL with 3 replicates on the Octet® RH96 BLI system with assay parameters: 400 rpm shake speed, 5 Hz acquisition rate, and 2 minutes assay read time, at 30°C. (B) hIgG standard calibration curve generated from triplicate samples and calculated using 4PL (unweighted) fitting model.

Materials Required

- Octet® BLI systems with Octet® BLI Discovery and Analysis Studio Software.
- Octet® ProA-MAX Biosensors, Sartorius Part No. 18-5184 (Tray), 18-5185 (Pack), & 18-5186 (Case).
- For all Octet® BLI Systems: Octet® 96FW Microplates, Sartorius Part No. 18-5172 (Pack), 18-5173 (Case).
- Optional for Octet® R8e, RH16 and RH96 BLI systems: Octet® 384TW Microplates, Sartorius Part No. 18-5166 (Pack), 18-5167 (Case).
- Purified standard protein (that is of the same molecule as the unknown samples) to be used as calibration standards.
- Octet® Sample Diluent (Sartorius Part No. 18-1104) for dilution of all samples.
- If crude samples need to be quantified, a "blank" (that is free of the molecules of interest) with the same matrix is required.

Assay Optimization Tips

The following optimization steps are recommended each time the quantitation assay involves a new matrix or a new hIgG protein.

- The calibration standard should be identical to the molecule analyzed in the unknown sample.
- The concentrations of the calibration standards should cover the expected range of the concentrations of the unknown samples.
- The matrices of the samples, standards, references, and biosensor hydration solution should be matched as closely as possible.
- Use a blank negative control in a matching matrix for background signal subtraction.
- If needed, determine the minimal dilution factor required to minimize matrix effect and achieve the targeted assay performance.

Dilution Factor Determination for Samples in Matrix

Components in complex matrices, such as cell culture media, can potentially interfere with assay performance. Before running a quantitation assay, empirically determine whether sample dilution is needed to minimize matrix effects. Diluting the sample matrix with the Octet® Sample Diluent (SD) can effectively reduce matrix-related interference. Dilution factor guidelines for various sample types are provided in Table 3.

Sample type	Matrix	Minimal dilution factor in SD
Human IgG	CHO-SFM media	Neat
Human IgG	DMEM	2-fold
Human IgG	DMEM with 10% FBS	2-fold
Human IgG	F12 Nutrient Mixture (HAM)	2-fold
Human IgG	Octet® Sample Diluent	Neat

Table 3. Recommended minimum dilution factors for IgG samples. Additional dilution may be required for crude samples in conditioned media with longer growth times, as these samples may contain protein or cell degradation products and aggregates that can interfere with IgG binding to Octet® ProA-MAX Biosensors. In all cases, the matrix for the standards, samples and the biosensor hydration solution should be matched as closely as possible.

1. Prepare 1 mL of each sample matrix (without target protein) diluted both 2-fold and 10-fold in the Octet® Sample Diluent.
2. Add target protein to the neat matrix, each of the matrix dilutions, and to the Sample Diluent as a control. The final concentration of target protein in each of the four samples should be in the middle of the desired quantitation range.
3. Transfer each sample to a 96- or 384-well sample plate in duplicate (eight wells total).
4. Hydrate biosensors in the sample matrix that match each sample type (e.g., biosensors to be used in wells with 10-fold diluted matrix should be hydrated in 10-fold diluted matrix).
5. Set up a Basic Quantitation assay according to the Octet® BLI Discovery Software User Guide.
6. Run the assay. Data will be displayed in real-time during the assay. Data and method files will be saved automatically to a location specified by user on the C: drive.
7. Load data into Octet® Analysis Studio Software.
8. Visually inspect the real-time binding traces and determine the dilution required to:
   a. Minimize non-specific binding of matrix components.
   b. Show equivalent binding in the matrix spiked sample and the Sample Diluent control.
9. Use this dilution factor for routine assays.

Assay Precision and Accuracy

To determine the quantitation range in any matrix, a precision and accuracy study should be carried out as follows:

1. Prepare a series of protein standards in the appropriate matrix diluent using the dilution factor determined in the Dilution Factor Determination for Sample Matrix experiment. The dilution series should span the entire range of the assay based upon the user experimental goal, such as 100–12000 µg/mL for assays run at 400 RPM.
2. Using the matrix diluent from Step 1, prepare two protein samples of known concentration for recovery measurement. The concentration of these samples should be within the range of the standard curve being generated, preferably one at the low end and one at the high end. These will be defined as unknown samples in the assay for calculating recovery.
3. Transfer triplicates of the prepared standards and the samples to a sample plate. It is recommended to organize samples from row A–H. Fill at least one well with a blank diluted matrix for reference subtraction during data analysis. An example plate map is shown in Figure 4.
4. Hydrate biosensors for 10 minutes in the matching matrix diluent.
5. Set up a Basic Quantitation assay using the same assay parameters that were used in the Dilution Factor Determination for Matrix experiment. Define sample Replicate Groups to calculate replicate averages and %CVs.
6. Run the experiment. Data will be displayed in real time during the assay. Data files, method files and assay pictures will be saved automatically.
7. Load the data into Octet® Analysis Studio Software.
8. If a blank matrix was included as a reference, use the reference subtraction option to correct the data as appropriate.
9. Calculate the binding rate. The results table will populate with calculated concentrations and data statistics.
10. Define assay dynamic range by selecting acceptable %CV values for the lower and upper concentration limits in the standard curve.
11. Exclude data points for the standard curve that lie outside the defined dynamic range if necessary.
12. Select the appropriate equation to fit the standard curve. Start the fitting with a 4- or 5-parameter logistic model, although other models may be used with suitable validation. Use a 5-parameter logistic equation for better recovery results if there are more lower concentration data points.
13. Evaluate the accuracy and precision of the assay using the calculated concentration value of the unknowns to determine % recovery and %CV.

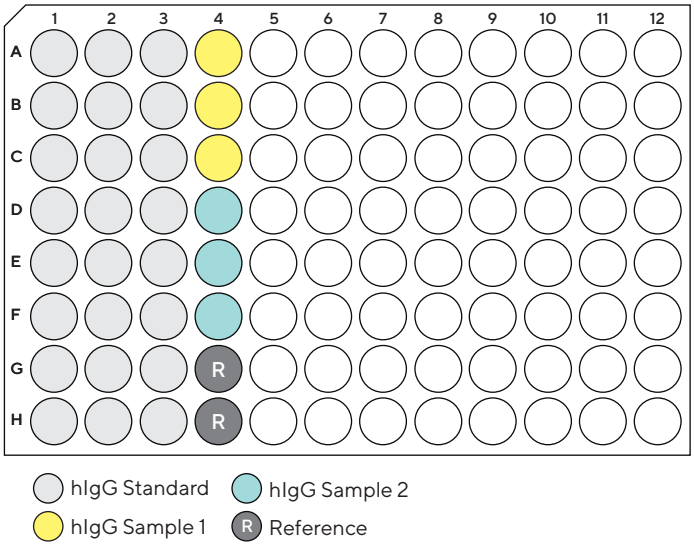

Figure 4. Example plate layout for a spike recovery assay.

Running the hIgG Quantitation Assay

- Prepare samples, calibration standards, and hydration solution according to the conditions determined in optimization steps in the prior section.
- Set up a Basic Quantitation assay using the parameters described previously in the optimization experiments. See Figure 5 for an example assay set up. Please note that standards can be run across the plate and not all at the beginning.
- Run the assay.
- Load data into Octet® Analysis Studio Software. Analyze as in previous optimization steps to determine the concentration of samples and data statistics.
- To export the analyzed data, click Save Report to generate a Microsoft® Excel® report.

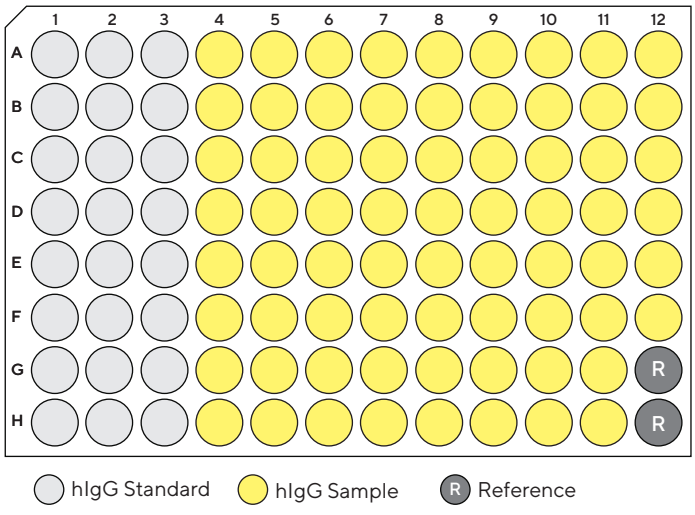

Figure 5. Example plate layout for a routine quantitation assay run on a 96-well microplate.

Regeneration of Octet® ProA-MAX Biosensors

The Octet® ProA-MAX Biosensors can be cost-effectively regenerated and reused up to 10 times in quantitation assays, either for generating replicate data or for analyzing large numbers of samples in high-throughput applications. Regeneration is performed by dipping the biosensors into a solution of 10 mM glycine at pH 1.7 for 5 seconds, followed by dipping them into the assay buffer for 5 seconds. These regeneration steps should be repeated 3–5 times in sequence to fully remove bound hIgG. After regeneration, the biosensors can be reused for quantitation of a new hIgG sample.

Regeneration results are sample-dependent. Users should determine the optimal regeneration conditions and the exact number of possible regeneration cycles experimentally, based on sample behavior and assay precision requirements. For an example quantitation assay with 10 regeneration cycles, see Figure 6 and Table 4.

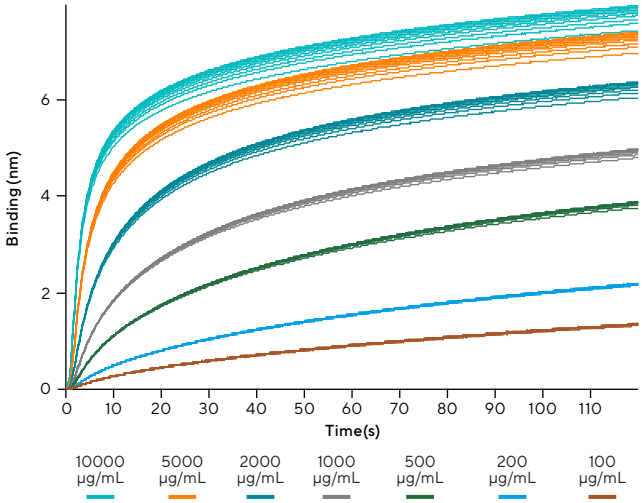

Figure 6. Overlay of binding curves for hIgG quantitation assay for concentration range of 100–10000 µg/mL after 10 regeneration cycles using 10 mM glycine with pH 1.7.

Known Titer µg/mL	Average Calculated Titer (10 Regenerations)	%CV (n=10)	%Recovery
10000	10059	2.4%	100.6%
5000	5000	1.9%	100.0%
2000	2033	1.0%	101.7%
1000	970.1	0.5%	97.0%
500	507.3	0.5%	101.5%
200	208.2	0.6%	104.1%
100	113.5	0.9%	113.5%

Table 4. Calculated titers and %CV for 10 regeneration cycles for hIgG quantitation assay.

Tips for Biosensor Regeneration

- Regenerate the Octet® ProA-MAX Biosensor surface after quantitation assay by dipping the biosensors into 10 mM Glycine, pH 1.7 for 5 seconds followed by neutralization in assay buffer for 5 seconds. Then repeat these regeneration steps 3–5 times.
- Depending on the analyzed sample and matrix, additional optimization of the regeneration buffer and/or conditions may be needed to achieve complete biosensor regeneration after each use.
- If biosensor regeneration is planned, it is recommended to pre-condition the biosensors before the first assay cycle to ensure the most consistent results. Pre-conditioning is performed by running the biosensor regeneration procedure once prior to use.
- It is important to ensure that the regeneration of biosensors for quantitation applications is complete. Quantitation results are significantly dependent on the surface capacity of the sensor. For example, a loss of 20% capacity over multiple regeneration cycles could affect the precision of quantitation by 10–20%.

Summary

Octet® ProA-MAX Biosensors demonstrate high specificity to hIgG and provide a wide dynamic range for hIgG quantitation (100–12000 µg/mL) while maintaining high accuracy and precision. Together, these features make them a reliable and versatile tool for titer measurement in both purified and crude high-titer samples across various stages of upstream and downstream bioprocessing workflows. A key advantage of these biosensors is their significantly increased upper detection limit compared with Octet® ProA Biosensors, which enables direct analysis of high-titer samples without serial dilution or purification, thereby simplifying workflows and reducing time to results. Like Octet® ProA Biosensors, Octet® ProA-MAX Biosensors can be regenerated and reused up to 10 times, making them a cost-effective solution for hIgG quantitation compared with other available technologies.

Ordering Information

Part No.	UOM	Description
18-5184	Tray	One tray of Octet® ProA-MAX Biosensors
18-5185	Pack	Five trays of Octet® ProA-MAX Biosensors
18-5186	Case	Twenty trays of Octet® ProA-MAX Biosensors

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