SARTORIUS

Octet® ProL Biosensors

For Quantitation of Whole-Molecule Antibody and Antibody Fragments

Technical Note

Scope
This technical note walks the reader through the use of Protein L coated biosensors in the optimal quantitation of various classes of kappa light chain containing antibodies.

Abstract

Octet® ProL Biosensors are coated with Protein L that has a high specificity for kappa light chain. Protein L binds to a more diverse set of antibodies and antibody fragments than Protein A and Protein G and is particularly useful when quantifying antibody fragments such as Fabs and single chain Fv molecules (ScFv). However, due to the high variability in amino acid sequence in the kappa light chain region of these molecules, the binding kinetics to Protein L, a principle used in the quantification process on Octet® Bio-Layer Interferometry (BLI) systems can differ; it is therefore important to use optimal standard samples for each type of molecule. The biosensors can be regenerated for re-use and have a dynamic range of quantitation of 0.05–2000 µg/mL.

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Overview

Octet® ProL Biosensors provide a rapid and direct method for quantifying a broad set of kappa light chain containing immunoglobulins, including whole molecules, FAb fragments and single chain variable fragments, in buffer, conditioned media or complex matrices. Protein L, which is factory-immobilized onto the biosensor, binds antibodies through the kappa light chain, and due to its specificity, recognizes a wider range of antibody classes than either Protein A or Protein G (Table 1, Figure 1) including IgG, IgM, IgA, IgE, and IgD. The ProL Biosensor is especially useful for quantifying antibodies and antibody fragments from serum-based cultures because Protein L does not bind bovine immunoglobulins, which often contaminate serum supplements.

Intended Use

The Octet® ProL Biosensor is intended for the detection and quantification of kappa light chain containing antibodies and antibody fragments in solution. Protein L is attached to the biosensor using a streptavidin-based technology but can be successfully used in the presence of media containing biotin, such as RPMI. If the biosensor is used in the presence of other biotinylated proteins, it is recommended to hydrate the biosensor in a matrix containing free biotin. All Sartorius consumable products are intended for research and manufacturing use only. They are not intended for diagnostic use in humans or animals.

Assay Principle

Protein L, originally isolated from Peptostreptococcus magnus, possesses high affinity for the kappa light chain of antibodies and antibody fragments. Immunoglobulin binding to the ProL Biosensor alters the interference pattern of light reflected from the biosensor surface, allowing the association event to be monitored in real time using the Octet® BLI system. Greater antibody concentrations result in faster binding rates. Unknown concentrations are determined by comparing real-time binding data to a standard curve constructed from samples of known concentrations of the same species and subtype.

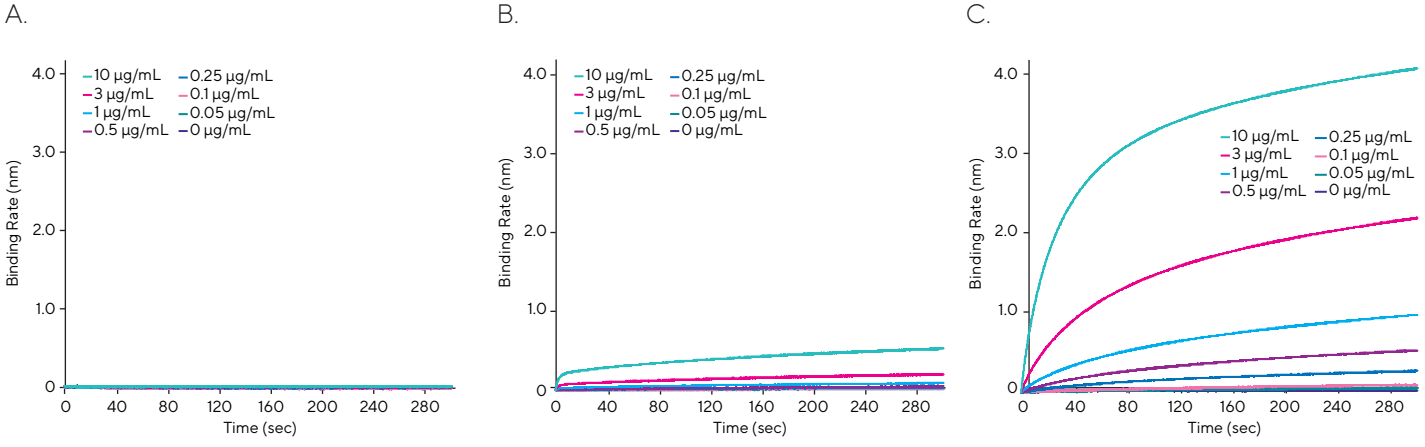
Due to its specificity for the kappa light chain, Protein L binds to a more diverse set of antibodies and antibody fragments than Protein A and Protein G. The Octet® ProL Biosensor is particularly useful for quantifying antibody fragments, such FAb and ScFv molecules, which cannot be detected with Protein A or Protein G.

The real-time data in Figure 1 compares the difference in binding of a human IgG FAb to ProL, ProA, and ProG Biosensors. The Octet® ProL Biosensor detects the hFAb at concentrations as low as 25 µg/mL, whereas Protein A and Protein G produce either negligible or very weak signals.

Analogous to the Protein A-Fc domain interaction, the Protein L and kappa light chain interaction can be disrupted using low-pH buffers. Because Protein L retains its binding activity after exposure to low pH, the biosensor can be

Figure 1

Differential Binding of a Human FAb Fragment to Octet® ProL, ProG, and ProA Biosensors.



Note. At 10 µg/mL, the human FAb fragment produced a strong signal of 4.0 nM on the Octet® ProL Biosensor (C). In contrast, on the Octet® ProA Biosensors (A) and Octet® ProG Biosensors (B) it produced weak signals of 0.01 and 0.5 nM, respectively. The ability of the ProL Biosensor to detect the kappa light chain moiety of an antibody expands the set of molecules addressable in an "out of the box" format using the Octet® BLI platform.

regenerated. Typically, a solution of 10 mM glycine at low pH (pH ~ 1.5) is used to dissociate the Protein L-kappa light complex and PBS (pH ~ 7.2) is used for neutralization. In general, good regeneration results often can be obtained using these standard conditions, but further optimization may also be required to maximize recovery of capacity.

Materials Required

- Octet® BLI system with Octet® Software version 6.1 or higher.
- Octet® ProL Biosensors (Sartorius part no. 18-5085 [tray]; 18-5086 [pack]; 18-5087 [case])
- For all Octet® BLI systems: 96-well, black, flat bottom, polypropylene microplate (Greiner Bio-One part no. 655209)
- Optional for Octet® RH16 and RH96 BLI systems:
  - 384-tilted well, black, flat bottom, polypropylene microplate (Sartorius part no. 18-5080 [pack]; 18-5076 [case])
  - 384-well, black, flat bottom, polypropylene microplate (GreinerBio-One part no. 781209)
  - Antibody to use as a calibration standard. For best results this calibration standard should be the same species and subtype as the samples to be quantified.
  - Octet® Sample Diluent (Sartorius part no. 18-1104) for dilution of all samples. If undiluted crude samples are to be quantified, a matching blank matrix is required.

Table 1
Recommended Minimum Dilution Factors and Calibration/Hydration Matrices for Common Sample Types.

Sample Matrix	Recommended Minimum Sample Dilution	Calibrator/ Control Matrix	Hydration Solution
Sample Diluent (SD)	None	SD	SD
CD OptiCHO	2X diluted in SD	2X diluted CD OptiCHO in SD	2X diluted CD OptiCHO in SD
RPMI	None	blank RPMI	blank RPMI
DMEM +10% FBS	None	blank DMEM + 10% FBS	blank DMEM + 10% FBS
CD DG44	None	blank CD DG44	blank CD DG44
FreeStyle 293	None	blank FreeStyle 293	blank FreeStyle 293

Tips for Optimal Performance

- Antibody species and subtypes have different binding kinetics to Protein L due to amino acid sequence variations and steric environments surrounding the binding site. Therefore, the species and subtype of unknown samples should match that of the standard samples for optimal performance.
- Typical assay sensitivity ranges from 0.05–300 µg/mL for assays run at 1000 rpm with a 5-minute read time and 0.5–2000 µg/mL for assays run at 400 rpm with a 2-minute read time. Both read time and shake speed can be adjusted to optimize the dynamic range if necessary.
- Match the matrix of the samples, standards, references, hydration solution and neutralization solution (if regenerating the biosensors) as closely as possible.
- Use reference subtraction of a blank negative control in a matching matrix for the most accurate quantitation of analytes with low concentrations.
- Fully equilibrate all reagents, calibrators and samples to room temperature prior to sample preparation. Thaw frozen samples completely and mix thoroughly prior to use.
- Hydrate the biosensors in a 96-well plate for a minimum of 10 minutes prior to use.
- Ensure that the Octet® BLI system is turned on and the lamp is warmed for at least 40 minutes prior to starting the assay.
- Set the sample plate temperature in the Octet® Software by selecting **Experiment > Set Plate Temperature**. Enter the desired temperature. Sartorius recommends 30 °C for accurate quantification. Set the default startup temperature (Software Version 6.4 and later), by selecting **File > Options**. Enter the desired temperature under Startup.

Assay Protocol

Overview

- Prepare the samples and calibration standards.
- Prepare the assay plate and biosensors.
- Run the experiment.
- Analyze the data.

Prepare the Samples and the Calibration Standards

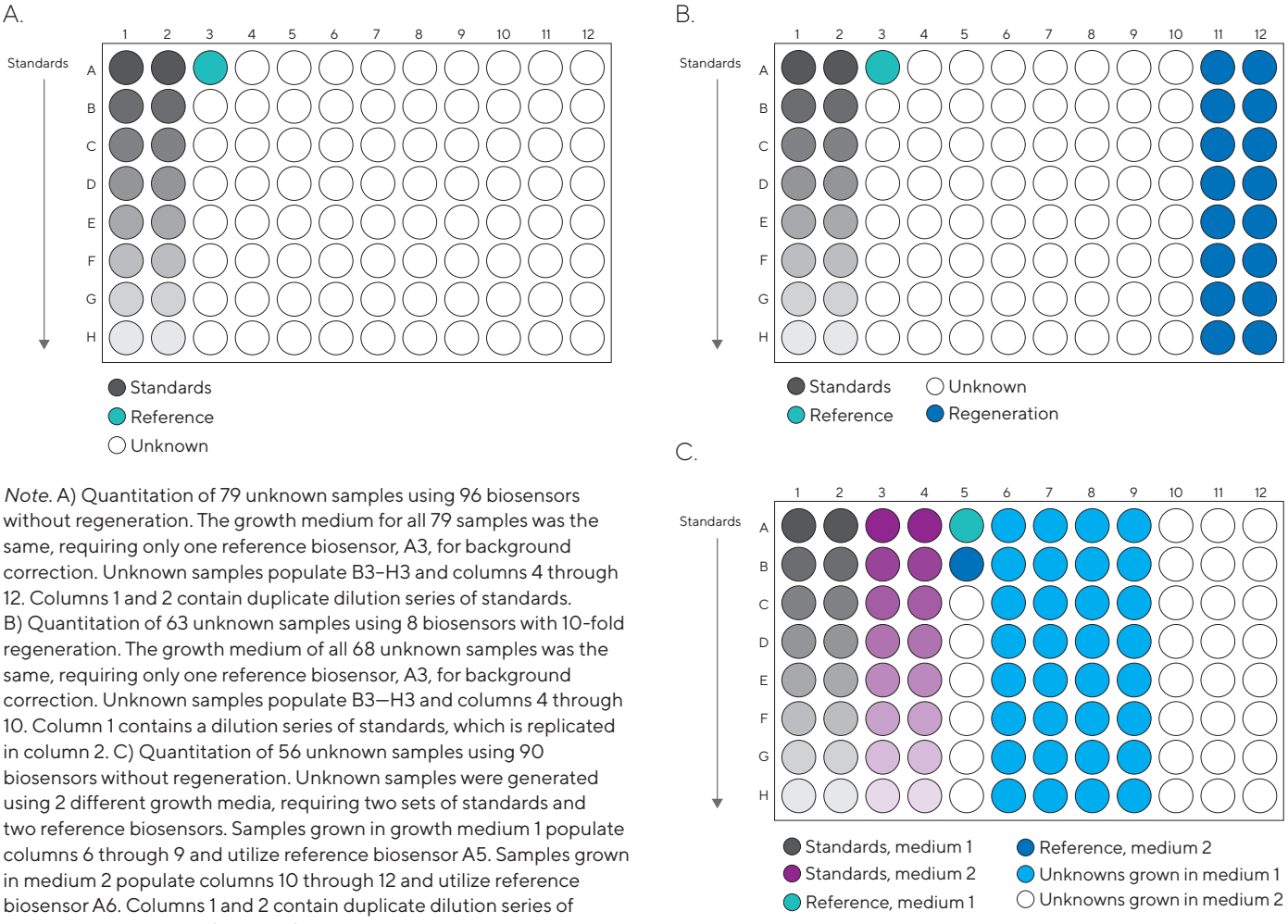
1. Samples, calibration standards and hydration solutions should be prepared according to the information in Table 1.
2. Minimum volume needed in each well for all samples, controls, calibrators and reagents:
   - 200 µL/well in a 96-well microplate (all Octet® BLI systems)
   - 80 µL/well in a 384-well microplate (Octet® RH16 and RH96 BLI systems only)
   - 40 µL/well in a 384-well, tilted bottom microplate (Octet® RH16 and RH96 BLI systems only)
3. Regenerating the biosensors requires a minimum of 2 mL of 10 mM glycine at pH 1.5 (or other pH determined to provide optimal regeneration for your assay).
4. Hydration requires a minimum of 200 µL of buffer for each biosensor. If regenerating the biosensor, the neutralization solution should be identical to the hydration solution, albeit at a different pH.

Prepare the Assay Plate and Biosensors

1. Pipette standards, controls and samples into a black flat-bottom microplate (see Figure 2 for examples of sample plate layouts).
2. Minimum volume in each well for all samples, controls, calibrators and reagents
   - 200 µL/well in a 96-well microplate (all Octet® BLI systems)
   - 80 µL/well in a 384-well microplate (Octet® RH16 and RH96 BLI systems only)
   - 40 µL/well in a 384-well, tilted bottom microplate (Octet® RH16 and RH96 BLI systems only)
3. If regenerating the biosensors, pipette regeneration solution and neutralization solution into wells as required by the assay protocol.
4. Pipette biosensor hydration solution into wells of a 96-well black flat bottom microplate corresponding to the number and position of the biosensors to be used.

Figure 2

Example Sample Plate Layouts for Antibody Quantitation of Unknown Samples Using ProL Biosensors.



Note. A) Quantitation of 79 unknown samples using 96 biosensors without regeneration. The growth medium for all 79 samples was the same, requiring only one reference biosensor, A3, for background correction. Unknown samples populate B3–H3 and columns 4 through 12. Columns 1 and 2 contain duplicate dilution series of standards. B) Quantitation of 63 unknown samples using 8 biosensors with 10-fold regeneration. The growth medium of all 68 unknown samples was the same, requiring only one reference biosensor, A3, for background correction. Unknown samples populate B3—H3 and columns 4 through 10. Column 1 contains a dilution series of standards, which is replicated in column 2. C) Quantitation of 56 unknown samples using 90 biosensors without regeneration. Unknown samples were generated using 2 different growth media, requiring two sets of standards and two reference biosensors. Samples grown in growth medium 1 populate columns 6 through 9 and utilize reference biosensor A5. Samples grown in medium 2 populate columns 10 through 12 and utilize reference biosensor A6. Columns 1 and 2 contain duplicate dilution series of standards in medium 1. Columns 3 and 4 contain duplicate dilution series of standards in growth medium 2.

Run the Experiment

1. Place the biosensor tray with the hydration plate in the Octet® BLI system. Place the sample plate (and reagent plate if applicable) in the Octet® BLI system. Warm the plate(s) in the instrument and hydrate the biosensors for 10 minutes prior to starting the experiment. The delay timer can be used to automatically start the assay after 10 minutes (600 seconds).
2. Set up a Basic Quantitation or a Basic Quantitation with Regeneration assay. For details on how to set up an assay see the Octet® Software User Guide. The dynamic range of the assay can be tuned by changing the shake speed and the read time (see Figure 3).
3. Run the assay.

Analyze the Data

1. Load data into Octet® Analysis Studio Software version 6.1 or later.
2. If blank matrix was included as a reference, use the reference subtraction option to correct the data as appropriate.
3. Analyze the data using the Initial Slope binding rate equation.
4. To export the analyzed data, use the Save Report button to generate a Microsoft® Excel® report.

Table 2

Human IgG Quantitation Results Using Different Assay Shake Speed and Read Time.

	400 rpm 2 Min Read Time		1000 rpm 5 Min Read Time	
Expected Conc. (µg/mL)	Avg. Conc. N=6 (µg/mL)	%CV (N=6)	Avg. Conc. N=6 (µg/mL)	%CV (N=6)
2000	2000.0	6.6%	–	–
1500	1508.8	3.8%	–	–
1000	1002.7	3.0%	–	–
700	700.7	1.3%	–	–
500	500.1	1.0%	–	–
300	301.0	2.1%	300.0	3.3%
100	100.0	1.2%	100.1	3.4%
30	30.0	2.8%	30.0	1.8%
10	10.0	1.6%	10.0	2.1%
3	3.0	3.9%	3.0	1.2%
1	1.0	1.8%	1.0	3.2%
0.5	0.5	4.2%	0.50	6.0%
0.25	–	–	0.25	6.4%
0.1	–	–	0.10	6.4%
0.05	–	–	0.05	6.6%

Table 3

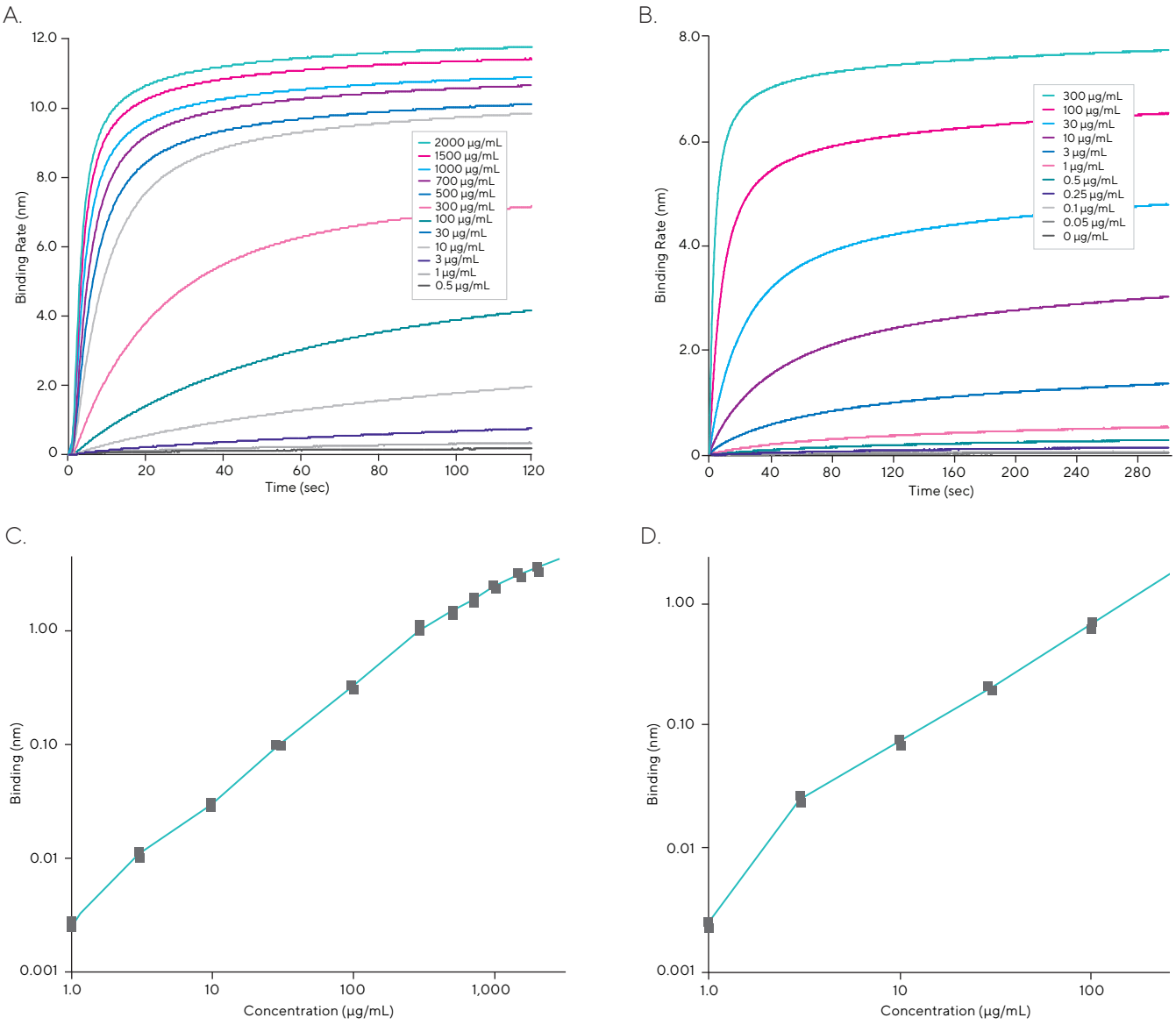
Eight ProL Biosensors were Used to Repeatedly Analyze Samples of 10 µg/mL Human IgG FAb in Neat CHO DG44 Medium at a Shake Rate of 400 rpm.

Biosensor #	Concentration (µg/mL) of hFAb Detected Per Regeneration Cycle										Statistics			
	R0	R1	R2	R3	R4	R5	R6	R7	R8	R9	Avg.	Std dev.	CV%	% Loss
1	10.2	10.1	10.0	10.0	10.0	10.0	9.91	9.87	9.92	9.85	9.99	0.11	1.1%	-3.4%
2	10.3	10.2	10.1	10.1	10.1	10.1	10.0	9.96	10.0	9.90	10.08	0.12	1.2%	-3.9%
3	10.4	10.3	10.3	10.3	10.2	10.2	10.2	10.1	10.1	10.1	10.22	0.10	1.0%	-2.9%
4	10.5	10.3	10.3	10.2	10.2	10.2	10.2	10.1	10.1	10.1	10.22	0.12	1.2%	-3.8%
5	10.1	10.0	10.0	9.96	9.86	9.83	9.80	9.77	9.70	9.67	9.87	0.14	1.4%	-4.3%
6	10.3	10.2	10.2	10.2	10.1	10.1	10.1	10.0	10.0	9.91	10.11	0.12	1.2%	-3.8%
7	10.1	10.1	10.1	10.0	9.97	9.89	9.90	9.84	9.80	9.72	9.94	0.13	1.4%	-3.8%
8	9.71	9.68	9.69	9.55	9.51	9.46	9.47	9.36	9.37	9.26	9.51	0.15	1.6%	-4.6%
									Overall		9.99	0.25	2.50%	-3.8%

Note. The ProL Biosensors were pre-conditioned with 3 regeneration cycles prior to the first measurement (R0) and regenerated in 10 mM Glycine pH 1.5 once between each subsequent measurement (R0 through R9). After 10 regeneration cycles, the average loss of capacity across the plate was less than 3.8% and the coefficient of variance across the plate was 2.5%, demonstrating efficient recovery of capacity and high reproducibility.

Figure 3

Examples of Dynamic Range with Different Flow Rates.



Note. Real-time binding curves of a human IgG standard assayed using ProL Biosensors on the Octet® R2 BLI system. The human IgG was assayed in Octet® Sample Diluent at the concentrations shown in the legends in µg/mL. A) Assay run at 400 rpm and 2 minutes read time. B) Assay run at 1000 rpm and 5 minutes read time. C) and D) are the resulting calibration curves from A) and B) respectively.

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