fortéBIO

Dip and Read™ Protein L Biosensors

For Quantitation of Antibodies and Antibody Fragments

OVERVIEW

Protein L 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 recognizes a wider range of antibody classes than either Protein A or Protein G, including IgG, IgM, IgA, IgE and IgD. Due to its specificity, Protein L recognizes mouse VkI (but not VkII, VkIII and VkIV) and human VkI, VkIII and VkIV (but not VkII). The Protein L 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 Protein L 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 biotinylated proteins, it is recommended to hydrate the biosensor in a matrix containing free biotin. All ForteBio 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 Protein L biosensor alters the interference pattern of light reflected from the biosensor surface, allowing the association and dissociation events to be monitored in real time using the Octet 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.

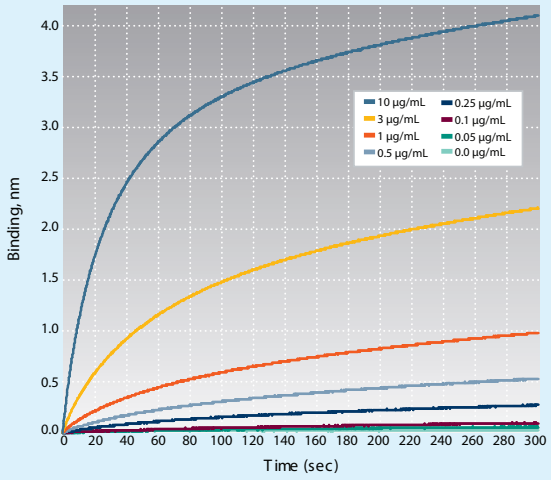
The real-time data in Figure 1 compares the difference in binding of a human IgG FAb to Protein L, Protein A and Protein G biosensors. The Protein L biosensor (graph A) detects the hFAb at concentrations as low as 50 ng/mL, whereas Protein G (graph B) and Protein A (graph C) produce either negligible or very weak signals.

Analogous to the Protein A-Fc domain interaction, the Protein L-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 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 sample buffer or matrix 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.

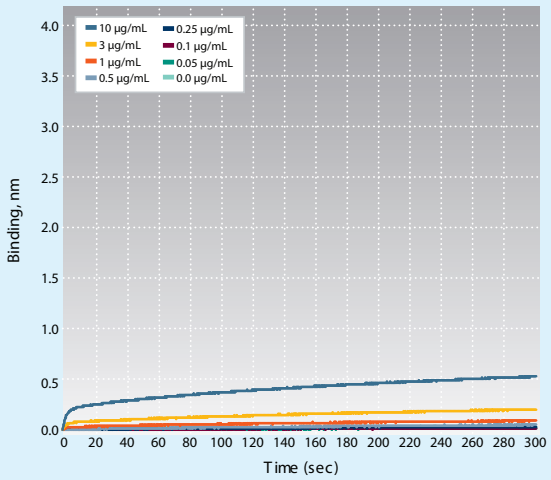
MATERIALS REQUIRED

- Octet instrument with Octet software version 6.1 or higher.
- Protein L biosensors (ForteBio part no. 18-5085 [tray]; 18-5086 [pack]; 18-5087 [case])
- For all Octet instruments: 96-well, black, flat bottom, polypropylene microplate (Greiner Bio-One part no. 655209)
- Optional for Octet RED384 and Octet QK384 instruments:
  - 384-tilted well, black, flat bottom, polypropylene microplate (ForteBio part no. 18-5080 [pack]; 18-5076 [case])
  - 384-well, black, flat bottom, polypropylene microplate (Greiner Bio-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.
- Sample Diluent (ForteBio part no. 18-5028) for dilution of all samples. If undiluted crude samples are to be quantified, a matching blank matrix is required.

A Protein L Biosensor



B Protein G Biosensor



C Protein A Biosensor

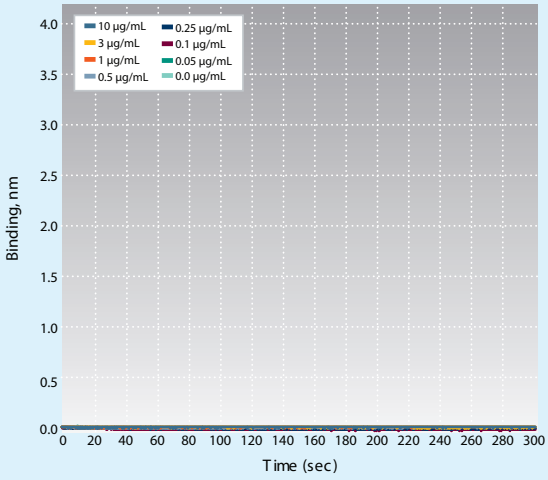


FIGURE 1: Differential binding of a human FAb fragment to Protein L (A), Protein G (B) and Protein A biosensors (C). At 10 µg/mL, the human FAb fragment produced a strong signal of 4.0 nm on the Protein L biosensor. In contrast, on the Protein A and Protein G biosensors it produced weak signals of 0.01 and 0.5 nm, respectively. The ability of the Protein L 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 platform.

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 a blank negative control in a matching matrix for reference subtraction. This is especially important when optimizing accuracy and detection of low-concentration analytes.
- 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 for a minimum of 10 minutes prior to use.
- Ensure that the Octet instrument 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. ForteBio 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"

Sample Matrix	*Recommended Minimum Sample Dilution*	*Calibrator/Control Matrix*	*Hydration Solution*
Sample Diluent (SD)	None	SD	SD
CD OptiCHO	2X 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

TABLE 1: Recommended minimum dilution factors and calibration/hydration matrices for common sample types.

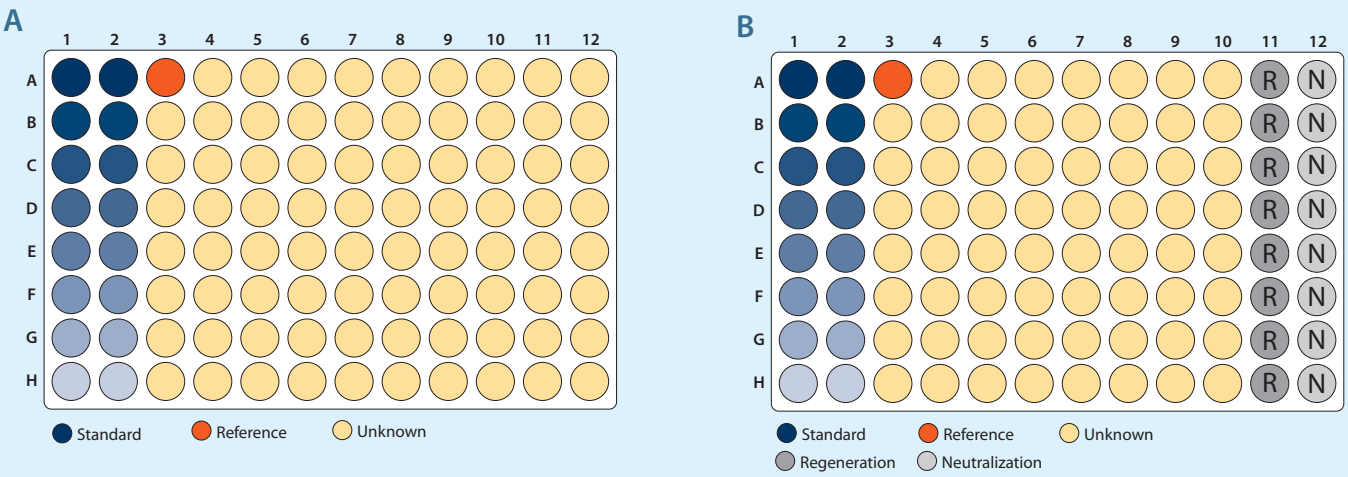


FIGURE 2: Two example sample plate layouts for antibody quantitation of unknown samples using Protein L Biosensors. **A**: Quantitation of 79 unknown samples using 96 biosensors without regeneration. The growth medium for all 79 samples was the same, requiring only one reference biosensor, A3, for background correction. Unknown samples populate B3–H3 and columns 4 through 12. Column 1 contains a dilution series of standards, which is replicated in column 2. **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. Column 11 contains a low pH regeneration buffer "R" that dissociates the antibody molecule from the Protein L biosensor surface and Column 12 contains a post-regeneration neutralization buffer "N".

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 instruments)
   - 80 µL/well in a 384-well microplate (Octet 384 instruments)
   - 40 µL/well in a 384-well, tilted bottom microplate (Octet 384 instruments 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).
4. Hydration requires a minimum of 200 µL of buffer for each biosensor. If biosensors are regenerated, then neutralization buffer should be identical to the hydration buffer and match the dilutions listed in Table 1.

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).

Expected Concentration (µg/mL)	Standard Sensitivity 400 rpm, 2-Minute Read Time		High Sensitivity 1000 rpm, 5-Minute Read Time	
	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 2: Average calculated concentration and %CV of replicates of human FAb calibration standards for the data from Figure 3. Assays for dynamic range and precision were performed with Sample Diluent as the matrix. Results may vary with changes in IgG species/subtype and assay matrix.

2. If regenerating the biosensors, pipette regeneration solution and neutralization solution into wells as required by the assay protocol.
3. 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.

Run the Experiment

1. Place the biosensor tray with the hydration plate in the Octet instrument. Place the sample plate (and reagent plate if applicable) in the Octet instrument. 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 Data Acquisition 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 Data Analysis 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.

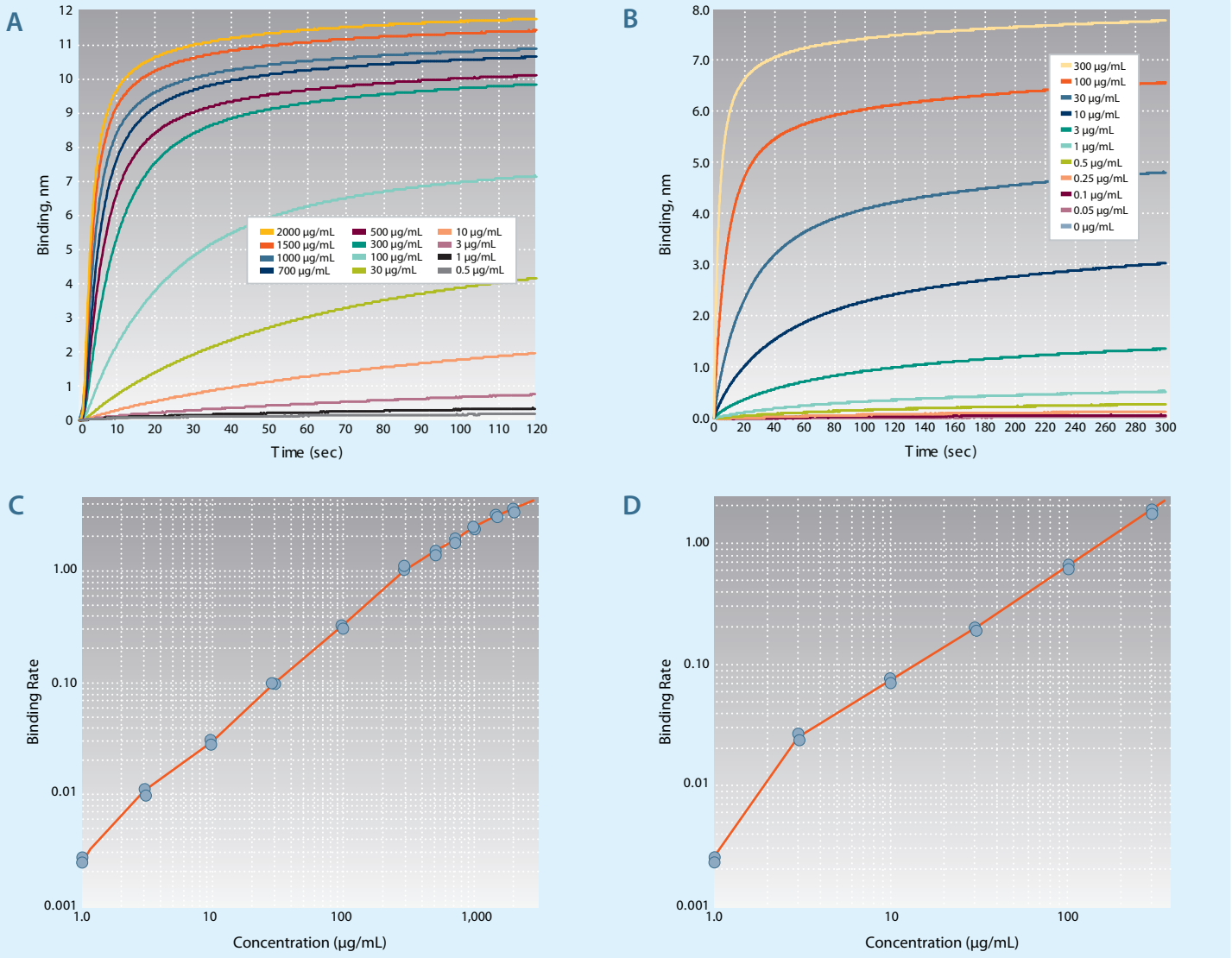


FIGURE 3: Detection of a human IgG standard using Protein L biosensors on the Octet RED system with standard (A,C) and high sensitivity (B,D) acquisition parameters. The human IgG was assayed in Sample Diluent at the concentrations shown in the legends in µg/mL. A) Assay run at 400 rpm and 2 minutes read time. B) Assay run at 1000 rpm and 2 minutes read time. C) and D) represent the resulting calibration curves from A) and B) respectively.

	Concentration (µg/mL) of hFAb Detected per Regeneration Cycle										*Statistics*			
Biosensor #	*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	10.1	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	10.2	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	10.2	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	10.1	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%

TABLE 3: Eight Protein L 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. The Protein L 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 3.8% and the coefficient of variance across the plate was 2.5%, demonstrating efficient recovery of capacity and high reproducibility.

ForteBio, Inc.
1360 Willow Road, Suite 201
Menlo Park, CA 94025
t: 888.OCTET-QK
or 650.322.1360

ForteBio, UK, Ltd.
83 Victoria Street, Suite 407
London, SW1H 0HW, UK
t: +44-(0)20-31784425

ForteBio (Aria Biotechnology Co. Ltd.)
917 Halley Road, Bldg 4
Zhangjiang High Tech Park
Shanghai, China 201203
t: +86-21-51320387

www.fortebio.com

P/N TN-3022 Rev B