fortéBIO

Protein G Biosensors

OVERVIEW

Protein G biosensors provide a rapid and direct method for quantification of many types of mammalian IgG from buffer, media or other complex matrices. Protein G, which is pre-immobilized onto the biosensor, binds to rodent species IgG and many other mammalian IgG with higher affinity than Protein A but does not bind IgM, IgD or IgA. While native Protein G will also bind to albumin, the recombinant form used in this product has the albumin and cell surface binding domains removed. This specificity for IgG makes Protein G biosensors useful for quantifying many species of IgG, including murine, goat, and bovine in the presence of other types of immunoglobulins or protein contaminants.

INTENDED USE

The Protein G Biosensor is intended for the detection and quantification of murine, goat, human and other IgGs from solution. The IgGs can be quantified from buffer or cell culture media with the appropriate dilution. The Protein G molecule is attached to the biosensor using a core streptavidin structure 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 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 G is an immunoglobulin binding protein isolated originally from the cell wall of the bacteria *G streptococci*. The Protein G on the biosensor is a recombinant form that has the albumin and cell surface binding domains removed. Thus, the Protein G biosensor will bind most mammalian IgGs specifically from solution samples. This binding can be monitored in real time using the Octet system. The real-time binding information is used to quantify the amount of IgG by comparing the binding rate of the unknown sample to the binding rates of known concentrations of the same species and subtype of IgG.

Protein G binds to many mammalian IgGs that bind poorly or not at all to Protein A. In particular, the Protein G biosensor is useful in quantifying murine, goat, and bovine IgGs that cannot be detected with Protein A. The real-time data in Figure 1 shows the difference in binding of various IgGs to Protein G biosensors compared to Protein A. The data in this figure also illustrates that the binding curve shapes, and thus binding kinetics, vary greatly depending on both the species and the subtype of IgG. For this reason it is critical that the IgG used as a calibration standard be the same species and subtype as the antibodies that are being quantified.

Since it is known that low pH buffers can disrupt the interaction between Protein G and IgG, the biosensor can be regenerated using a 10 mM Glycine at a low pH (typically pH 1.7–2). In general, good regeneration results can be obtained using 10 mM Glycine at pH 1.7 but in some cases the pH may need to be optimized further in order to regenerate the binding capacity of the sensors completely.

MATERIALS REQUIRED

- Octet Instrument with Octet software 4.0 or higher.
- Protein G biosensors (ForteBio part no. 18-5082 [tray]; 18-5083 [pack]; 18-5084 [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:

  - 384-well, tilted-bottom, black polypropylene microplate (ForteBio part no. 18-5080 [pack]; 18-5076 [case])
  - 384-well, black, flat bottom, polypropylene microplate (Greiner Bio-One part no. 781209)
- IgG 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.

TIPS FOR OPTIMAL PERFORMANCE

Different species and subtypes of IgG will have different binding kinetics to Protein G due to differing sequences and steric environments surrounding the binding site. As such, matching species and subtype, and therefore the binding kinetics, of the standards and the unknown samples is required for optimal performance.

- Typical assay range measured in sample diluent is 300–0.05 µg/mL for assays run at 1000 rpm with a 5 minute read time and 2000–0.5 µg/mL for assays run at 200 rpm with a 2 minute read

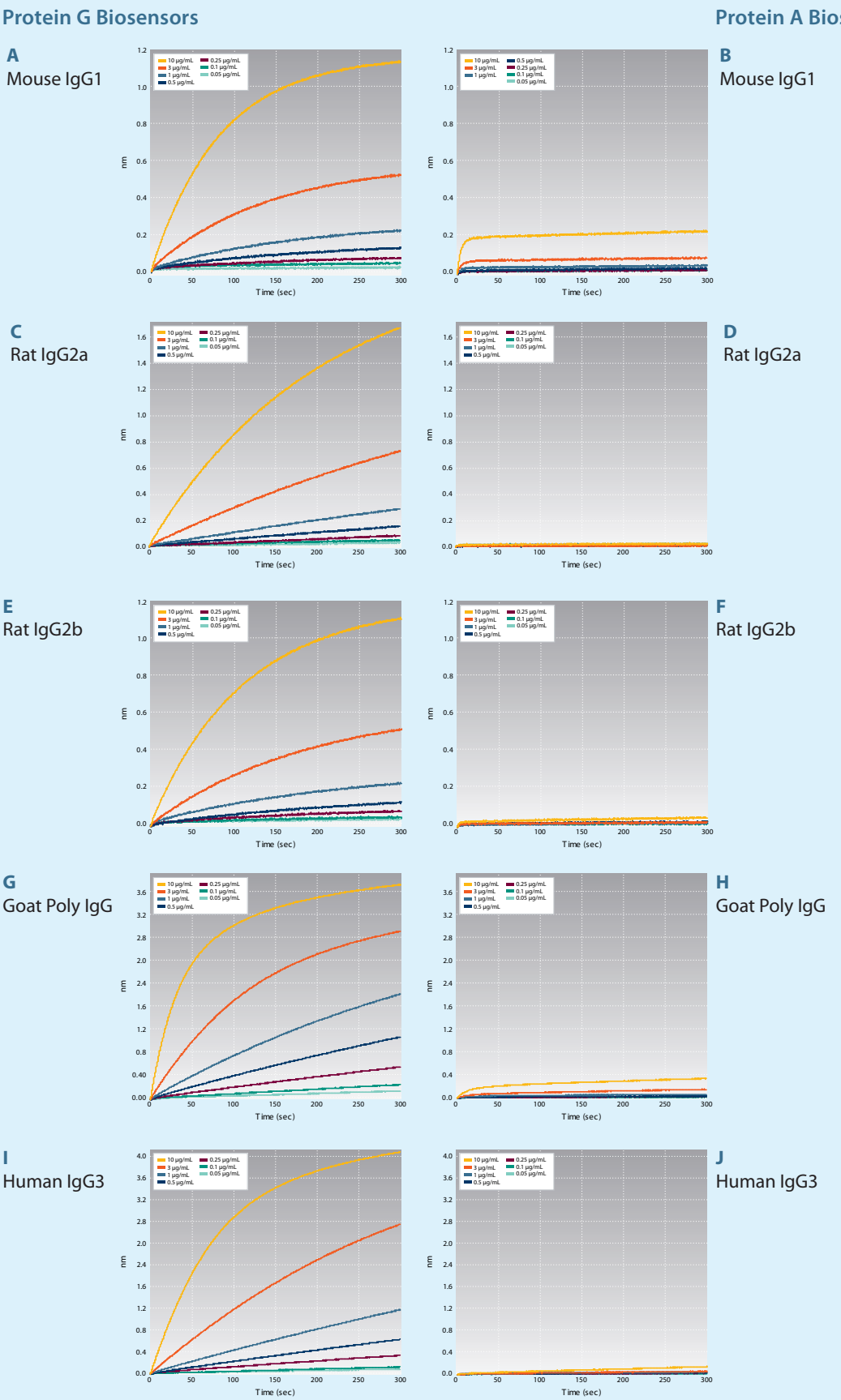

FIGURE 1: Differences in binding for several species and subtypes of IgG on Protein G biosensors (A,C,E,G,I) and Protein A biosensors (B,D,F,H, J). Each assay was run at 1000 rpm and 5 minutes read time. The concentration of each IgG is shown in the chart legend in µg/mL.

time. Both read time and shake speed can be adjusted to optimize the dynamic range if needed.

- Use a calibration standard that is the same IgG as the one contained in the unknowns.
- Match the matrix of the samples, standards, references and hydration solution as closely as possible. Use reference subtraction of a blank negative control in matching matrix for the most accurate quantitation of low concentrations.
- Fully equilibrate all reagents, calibrators and samples to room temperature prior to sample preparation. For frozen samples, thaw 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 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 File > Experiment > Set plate temperature. Enter the desired temperature. ForteBio recommends 30°C for accurate quantitation.

ROUTINE ASSAY USE

Overview

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

Prepare the Samples and the Calibration Standard

1. Samples, calibrator standards and hydration solutions should be prepared according to the information in Table 1.
2. Minimum volumes for samples, controls, calibrators and reagents:
   - a. 200 µL/well in a 96-well microplate (all Octet instruments).
   - b. 80 µL/well in a 384-well microplate (only when using Octet 384 instruments)
   - c. 40 µL/well in a 384-well, tilted bottom microplate (only when using Octet 384 instruments)
3. If regenerating the biosensor, a minimum of 2 mL of the 10 mM Glycine at pH 1.7 (or other pH identified) is needed.
4. A minimum of 200 µL of hydration solution is needed for each biosensor. If regenerating the biosensor, the neutralization solution should be identical to the hydration solution.

Prepare the Assay Plate and Biosensors

1. Pipette standards, controls and samples into a black flat bottom microplate (see Figure 2 for an example of a sample plate layout).
2. Minimum volumes for samples, controls, calibrators and reagents:
   - a. 200 µL/well in a 96-well microplate (all Octet instruments).
   - b. 80 µL/well in a 384-well microplate (only when using Octet 384 instruments).
   - c. 40 µL/well in a 384-well, tilted bottom microplate (only when using Octet 384 instruments).

Sample Matrix	*Minimal Sample Dilution*	*Calibrator/Control Matrix*	*Hydration Solution*
Buffer	neat	buffer	buffer
	1 to 10 in SD*	SD*	SD*
CD-CHO (serum-free)	neat	blank CD-CHO	blank CD-CHO
	1 to 10 in SD*	SD*	SD*
RPMI (serum-free)	neat	blank RPMI	blank RPMI
	1 to 10 in SD*	SD*	SD*
DMEM +10% FBS	1 to 10 in SD*	SD*	SD*

**SD = Sample Diluent*

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

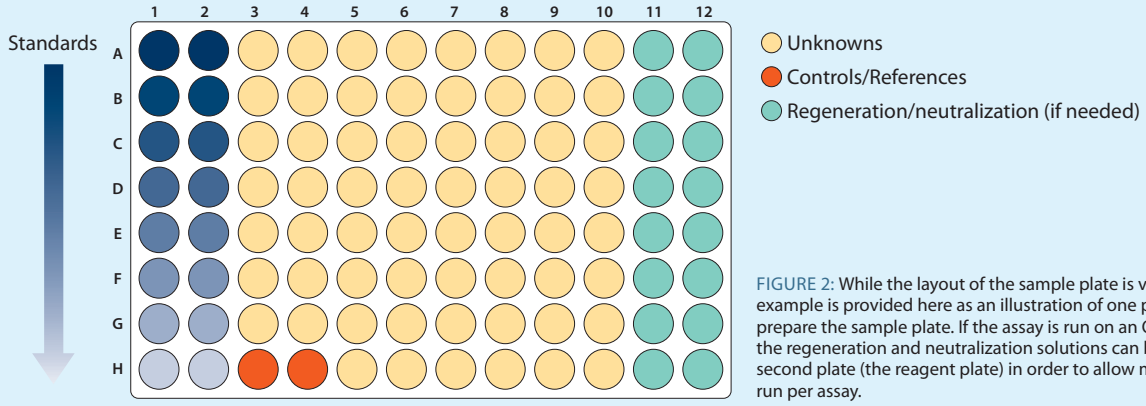

FIGURE 2: While the layout of the sample plate is very flexible, an example is provided here as an illustration of one possible way to prepare the sample plate. If the assay is run on an Octet 384 instrument, the regeneration and neutralization solutions can be moved to the second plate (the reagent plate) in order to allow more samples to be run per assay.

3 If regenerating, 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.

Run the Experiment

1 Place the biosensor tray with the hydration plate into the Octet instrument. Place the sample plate (and reagent plate if applicable) into the Octet instrument. Warm the plates in the instrument and hydrate the biosensors for 10 minutes prior to the start of 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 4.0 or later.

2 If blank matrix was included as a reference, use the reference subtraction option to correct the data as appropriate (reference subtraction only available in Data Analysis 6.1 or later).

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.

EXAMPLE DATA

Example data are shown in Figure 3 and Table 2, below.

DISCLAIMER

ForteBio reserves the right to change its products and services at any time to incorporate the latest technological developments. This technical note is subject to change without notice.

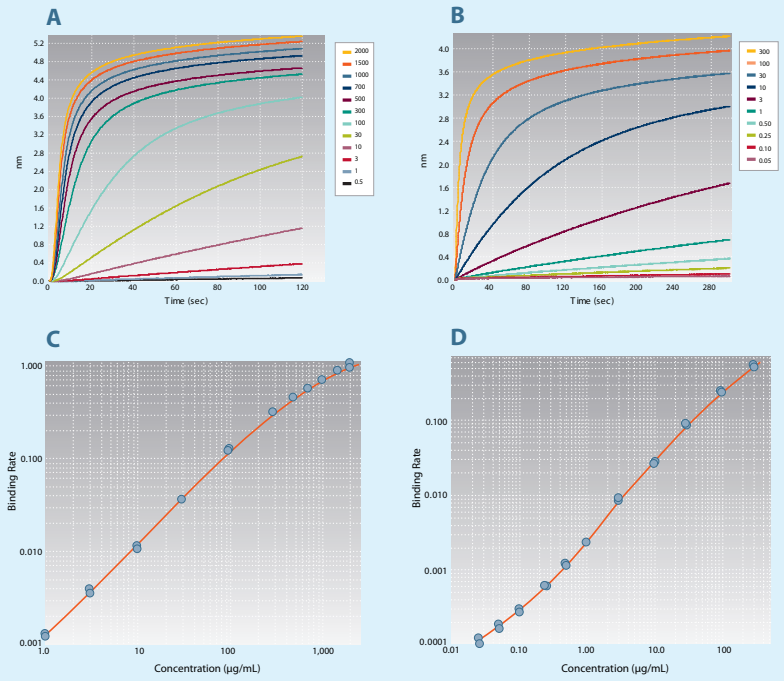

FIGURE 3: A) and B) Real-time binding curves of a polyclonal mouse IgG calibrator standard assayed using Protein G biosensors on the Octet RED system. The mouse IgG was assayed in Sample Diluent at the concentrations shown in the legends in µg/mL. A) Assay run at 200 rpm and 2 minutes read time. B) Assay run at 1000 rpm and 5 minutes read time. C) and D) are the resulting calibration curves from A) and B) respectively.

	200 rpm 2 min read time		*1000 rpm 5 min read time*	
Expected conc (µg/mL)	*Ave conc N=5 (µg/mL)*	*%CV N=5*	*Ave conc N=4 (µg/mL)*	*%CV N=4*
2000	2157.3	9%	—	—
1500	1481.6	3%	—	—
1000	958.2	2%	—	—
700	696.9	2%	—	—
500	485.5	1%	—	—
300	308.3	1%	01.0	2%
100	105.6	1%	100.1	1%
30	29.9	1%	30.1	2%
10	9.3	2%	10.0	1%
3	3.2	5%	3.0	5%
1	1.0	2%	1.0	1%
0.50	0.5	3%	0.50	2%
0.25	—	—	0.25	3%
0.10	—	—	0.10	4%
0.05	—	—	0.05	8%

TABLE 2: Average calculated concentration and %CV of replicates of mouse IgG calibration standards for the data from Figure 3. Dynamic range and precision shown are from polyclonal mIgG in Sample Diluent and may vary with changes in IgG species/subtype or changes in assay matrix.

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