SARTORIUS

Octet® GST Biosensor Quantitation Assays

Technical Note

Scope
This technical note provides guidance on the use of Octet® GST Biosensors for rapid label-free quantitation of GST-tagged proteins.

Abstract

Detection and quantitation of recombinant proteins especially in crude lysates is often challenging as traditional protein concentration measurement techniques such as measuring UV absorbance at 280 nm require purified samples. Octet® GST Biosensors specifically bind to GST-tagged proteins and enable users to circumvent protein purification when determining concentration through a direct one-step dip into crude or cell lysates. Depending on assay setup, the biosensor can be used with up to 2000 µg/mL of protein.

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Overview

Glutathione-S-transferase (GST) is commonly fused to recombinant proteins as a means of facilitating detection, purification and increasing solubility. The Octet® GST Biosensor consists of a high affinity anti-GST antibody pre-immobilized on a Sartorius biosensor. In conjunction with the Octet® BLI system, the GST Biosensor provides a rapid and label-free method for GST-tagged protein quantitation and kinetic analysis. The high specificity of the antibody-based biosensor enables direct analysis of GST analytes in crude lysates, column eluents, cell lysates and cell culture supernatants, serving as a time-saving alternative to traditional analytical methods. For more information on kinetic analyses using the GST Biosensor, please see the Octet® GST Biosensor Kinetic Assays Technical Note.

Principle

GST Biosensors are fiber optic devices coated with a polyclonal anti-GST antibody for the quantitation of GST fusion protein analytes. The binding of the molecules to the biosensor alters the interference pattern of light reflected from the surface, allowing binding events to be monitored in real time using the Octet® BLI platform. Higher analyte concentrations result in both faster binding rates and larger signal amplitudes. Unknown concentrations are determined by comparing either kinetic (binding rate) or equilibrium (signal amplitude) data to a standard curve constructed from identical samples of known concentrations.

Materials Required

- Octet® BLI system with Octet® Software
- GST Biosensors (Sartorius part no. 18-5096 [tray]; 18-5097 [pack]; 18-5098 [case])
- For all Octet® BLI systems: 96-well, black, flat bottom, polypropylene microplate (Greiner Bio-One part no. 655209) or Sartorius approved sample plates
- 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 (Greiner Bio-One part no. 781209)
- Standard protein containing a GST tag to be used as a calibration standard. For best results this calibration standard protein should be identical to the protein in the sample
- 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

Tips for Optimal Performance

- GST-tagged analytes will possess different binding kinetics due to amino acid sequence variations and differing steric environments. Since the quantitation performance is typically based on binding kinetics, the calibration standard protein should be identical to the GST protein present in the unknown sample for best results.
- Typical assay sensitivity ranges from 0.25–2000 µg/mL for assays run at 400 rpm with a 2-minute read time and 0.10–200 µg/mL for assays run at 1000 rpm with a 2-minute read time.
- Match the matrix of the samples, standards, references, and hydration solution as closely as possible.
- Perform a dilution study and a dynamic range study as outlined in the Assay Optimization section.
- Use a blank negative control in a matching matrix for reference subtraction. This is especially important when optimizing accuracy and detecting 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 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. Sartorius recommends 30°C for accurate quantitation. Set the default startup temperature (software version 6.4 and later) by selecting **File > Options**. Enter the desired temperature under Startup.
- The GST Biosensor is constructed using elements of the protein streptavidin. For biotinylated analytes or matrices that contain biotinylated molecules, it is recommended that potentially open biotin binding sites be blocked by incubating the biosensors in 15 µg/mL of biocytin for 15 minutes.

Assay Optimization

The following assays are recommended each time a new matrix or new GST-tagged protein is analyzed.

Overview

1. Determine the minimal dilution factor required to achieve the targeted assay performance.
2. Perform a spike/recovery study to determine assay dynamic range.
3. Determine data analysis parameters.
4. Apply finalized protocol and data analysis parameters in routine assay.

Dilution Factor Determination for Matrix

Differences between matrices can potentially influence assay performance. Diluting the sample matrix using Sartorius' Sample Diluent is a convenient and often effective means of minimizing matrix effects. It is therefore recommended to determine the minimum dilution factor using Sample Diluent that achieves the desired assay performance.

1. Prepare 2 mL of sample matrix diluted both two-fold and ten-fold in Sample Diluent. General guidelines for dilutions are described in Table 1.
2. Prepare a spiked sample of the GST-tagged protein to be quantitated in: Sample Diluent, neat matrix, two-fold diluted matrix and ten-fold diluted matrix by mixing the minimum volume of GST analyte and 0.5 mL of each matrix (four samples total). The final concentration of the GST-tagged protein should be in the middle of the desired quantitation range.
3. Transfer each sample to a 96-well or 384-well sample plate in duplicate (eight wells total).
4. Hydrate biosensors in the sample matrix that matches each sample type (e.g., biosensors to be used in wells with ten-fold diluted matrix should be hydrated in ten-fold diluted matrix). Place the sample plate and the hydrated biosensors into the instrument. Recommended sample plate warm-up in the instrument and biosensor hydration time is 10 minutes. The delay timer can be used to automatically start the assay after 600 seconds.
5. Set up a Basic Quantitation assay according to the Octet® Software User Guide. Use one of the provided method template files for the GST Biosensor or use the **Modify** button in the Plate Definition tab to choose the appropriate anti-GST parameter file (parameter file availability will depend on software version number).
   a. **GST Biosensor – Standard range:** Loads parameters for a 120-second assay at 400 rpm, typically suited for a 0.25 µg/mL to 2000 µg/mL dynamic range (varies with GST analyte and sample matrix).
   b. **GST Biosensor – High Sensitivity:** Load parameters for a 120-second assay at 1000 rpm, typically suited for a 0.1–200 µg/mL range (varies with GST analyte and sample matrix).
6. Run the experiment.
7. Data will be displayed in real time during the assay. Data and method files will be saved automatically.
8. Load data into Octet® Analysis Studio or Data Analysis Software.
9. Visually inspect the real-time binding traces and determine the dilution required to:
   a. Minimize non-specific binding from the matrix.
   b. Show equivalent GST analyte binding in the matrix spiked sample and the Sample Diluent control.
10. Use this dilution factor for routine assays.

Table 1
Recommended Minimum Dilution for Common Sample Types.

Sample Type	Minimum Recommended Dilution in Sample Diluent
Purified proteins	Dilute into assay range
Samples from column eluents	Dilute into assay range
Serum free cell culture supernatants media	Neat or two-fold
Serum containing cell culture supernatants	Neat
Bacterial cell pellet lysed by sonication	Neat
Bacterial cell pellet lysed by sonication	Fifty-fold

Note. In all cases the matrix for the diluted samples, the standards and the biosensor hydration solution should be matched as closely as possible.

Spike Recovery Assay

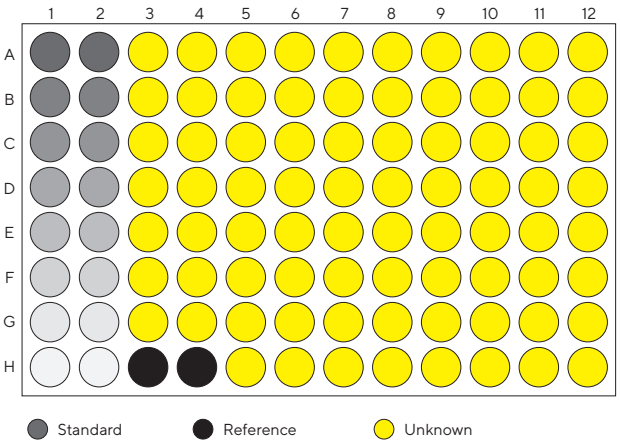
To determine the dynamic range and data analysis parameters suitable for a specific GST analytes, establish a standard curve and spike recovery as described below.

1. Prepare a series of GST standards in the matrix using the dilution factor determined above. The typical range of the series spans 0.25–2000 µg/mL for the Standard Range Assay at 400 rpm and 0.10–200 µg/mL for the High Sensitivity Assay at 1000 rpm. A minimum volume of 200 µL/well in a 96-well microplate, 80 µL/well in a 384-well microplate or 40 µL/well in a 384-well tilted bottom microplate is required. Recommended concentrations for the standard curve are described in Figure 2.
2. Using the same sample matrix as in Step 1, prepare 700 µL of two unknown samples. The concentration of these samples should be within the assay dynamic range.
3. Transfer triplicates of the standards and the unknowns to a sample plate. It is recommended to organize samples in columns, from A–H. Fill at least one well with blank diluted matrix for reference subtraction during data analysis. An example plate map is shown in Figure 1.
4. Hydrate biosensors in the matrix that matches the blank diluted matrix. Place the sample plate and the hydrated biosensors in the Octet® BLI system. The recommended sample plate warm-up in the instrument and biosensor hydration time is 10 minutes. The delay timer can be used to automatically start the assay after 600 seconds.
5. Set up a Basic Quantitation assay using either the Standard or High Sensitivity Assay parameters that were used in the matrix dilution assay above. Availability of the pre-loaded GST assay parameters will be software version-dependent.
6. Run the experiment. Data will be displayed in real time during the assay. Data files, method files and assay pictures (.jpgs) will be saved automatically.
7. Load the data into Octet® Analysis Studio Software.
8. If blank matrix was included as a reference, use the reference subtraction option to correct the data as appropriate.
9. Calculate the binding rate.
10. Define a dynamic range by selecting acceptable % CV values for the lower and upper concentration limits. 10% is routinely used as a threshold, but may vary depending on the requirements of each assay.
11. Exclude data points for the standard curve that lie outside the defined dynamic range.
12. Iteratively adjust the following processing parameters and re-calculate the binding rate:
    a. Adjust the read time window if necessary (typically 120 seconds).
    b. Adjust the zero concentration threshold if necessary (recommended 0.0001).
    c. Adjust the low concentration threshold to 0.001 (recommended).
    d. Select the appropriate standard curve equation.
13. Evaluate the calculated concentration value of the unknowns by defining acceptable values of % recovery (accuracy) and % CV (precision). ± 15% recovery and 10% CV are frequently used threshold values but may vary depending on the requirements of each assay. See Figure 2 and Table 2 for representative data at 400 rpm and 1000 rpm.

Assay Protocol

1. Prepare samples, calibration standards and hydration solutions according to the information contained in Table 1. The minimum volume needed in each well varies with the plate used:
   - 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)
   - 40 µL/well in a 384-well, tilted bottom microplate (Octet® RH16 and RH96 BLI systems only).
2. Pipette standards, controls and samples into a black polypropylene microplate (see Figure 1 for a sample plate layout).
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.

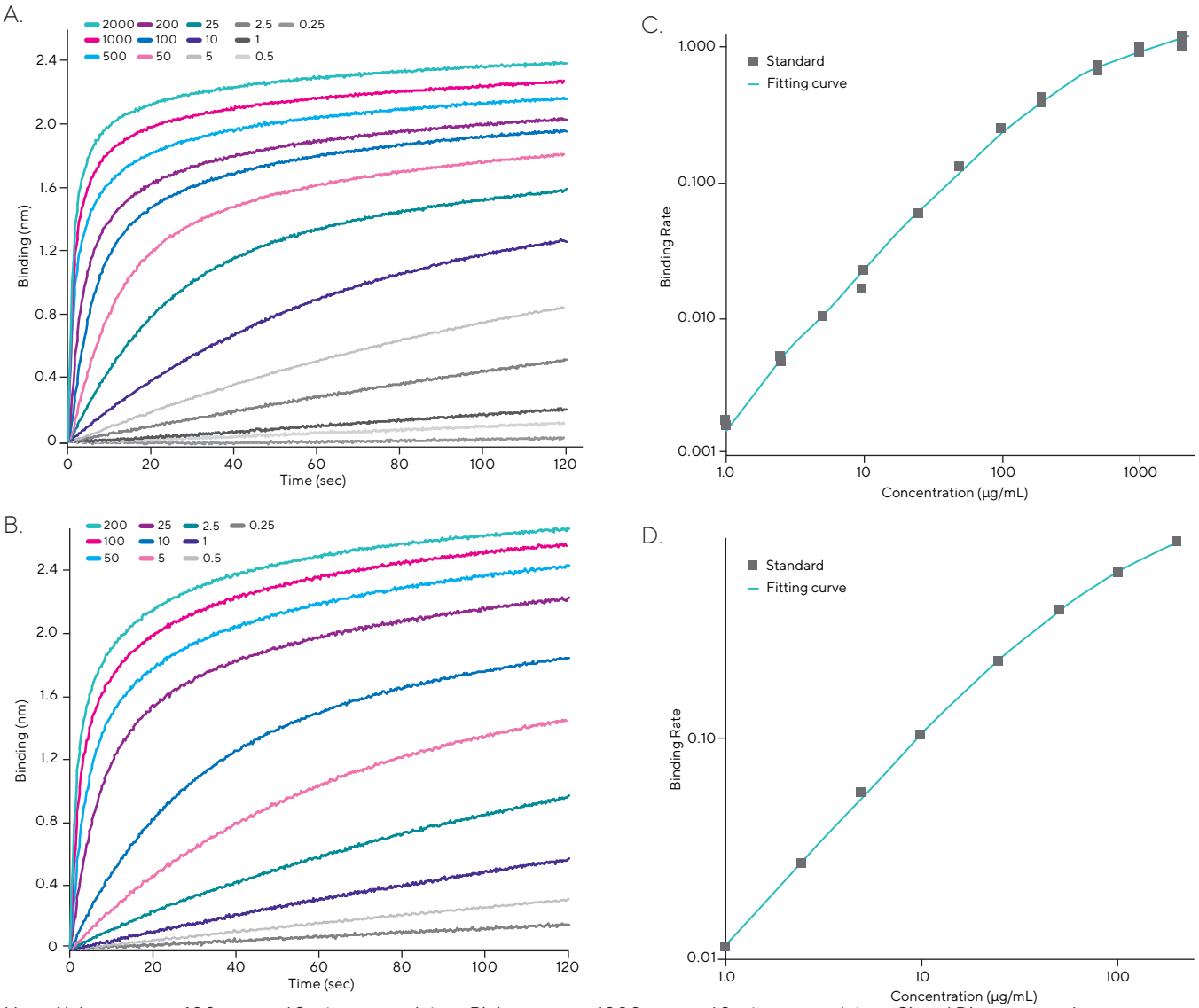
Figure 1
Example Plate Layout for a Routine Assay Run in a 96-well Microplate.



4. Place the biosensor tray with the hydration plate in the instrument. Place the sample plate in the instrument. Warm the sample plate 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).
5. Set up a Basic Quantitation assay. An example plate map is shown in Figure 1. 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 2).
6. Run the assay.
7. Load data into Octet® Analysis Studio Software.
8. If blank matrix was included as a reference, use the reference subtraction option to correct the data as appropriate.
9. Calculate the binding rate.
10. Define a dynamic range by selecting acceptable % CV values for the lower and upper concentration limits. 10% is routinely used as a threshold, but may vary depending on the requirements of each assay.
11. Exclude data points for the standard curve that lie outside the defined dynamic range.

Figure 2

Detection of a Ubiquitin-GST Standard Using GST Biosensors on the Octet® RH16 BLI System with Assay Parameters for a Standard Dynamic Range (A, C) and High Sensitivity Dynamic Range (B, D).



Note. 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. Sample diluent was used as a matrix for all samples.

12. Iteratively adjust the following processing parameters and re-calculate the binding rate:
    a. Adjust the read time window if necessary (typically 120 seconds).
    b. Adjust the zero concentration threshold if necessary (recommended 0.0001).
    c. Adjust the low concentrations threshold to 0.001 (recommended).
    d. Select the appropriate standard curve equation.
13. To export the analyzed data, use the save report button to generate a Microsoft® Excel® report.

Representative Data

Figure 2 shows detection of a Ubiquitin-GST standard using Octet® GST Biosensors on the Octet® RH16 BLI system with assay parameters for a standard dynamic range (A, C) and high sensitivity dynamic range (B, D). A) Assay run at 400 rpm and 2-minute read time. B) Assay run at 1000 rpm and 2-minute read time. C) and D) represent the resulting calibration curves from A) and B) respectively. Sample diluent was used as a matrix for all samples. See Table 2 for the statistical analysis of data from Table 1.

Table 2

Average Calculated Concentration and %CV of Triplicates of Ubiquitin-GST Calibration Standards for the Data from Figure 2.

	Standard Range 400 rpm 2 Min. Read Time		High Sensitivity 1000 rpm 2 Min. Read Time	
Expected Concentration (µg/mL)	Avg. Conc. µg/mL (N = 3)	% CV (N = 3)	Avg. Conc. µg/mL (N = 3)	% CV (N = 3)
2000			2000.0	4.4%
1000			1009.4	4.4%
500			507.4	4.6%
200	200.0	2.5%	201.5	3.8%
100	100.6	3.3%	100.3	2.5%
50	50.6	6.5%	50.0	0.8%
25	25.1	4.3%	25.0	0.4%
10	10.0	4.7%	10.0	1.3%
5	5.01	6.2%	5.00	0.4%
2.5	2.5	5.0%	2.50	2.0%
1	1.00	3.5%	1.00	4.9%
0.5	0.50	5.0%	0.50	3.0%
0.25	0.25	0.8%	0.25	2.3%
0.1	0.10	6.2%		
0.05	0.05	10.2%		

Note. Results may vary with individual GST analytes and assay matrices.

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