GST biosensor kinetics assays

Overview

Glutathione-S-transferase (GST) is commonly fused to recombinant proteins to facilitate detection and purification, and to increase solubility. The anti-GST biosensor consists of a high affinity anti-GST antibody pre-immobilized on a ForteBio biosensor. In conjunction with the Octet® System, the anti-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 the direct quantitation of GST analytes in crude lysates, column eluents, cell lysates and cell culture supernatants, serving as an alternative to traditional time-consuming analytical methods. For more information on quantitation analyses using the anti-GST biosensor, please see the GST Quantitation Technical Note.

Principle

Anti-GST biosensors are fiber optic devices coated with a polyclonal anti-GST antibody for detection of interactions between GST fused proteins and biomolecular analytes. This antibody can capture and immobilize GST analytes for both quantitation and kinetic applications. The binding of the target analytes to immobilized GST fusion proteins alters the interference pattern of light reflected from the biosensor surface, allowing molecular association and dissociation events to be monitored in real-time using the Octet instrument platform. The surface is well suited for capture and analysis directly from complex mixtures as an alternative to chemical protocols such as EDC/NHS and biotinylation.

Anti-GST biosensors can be regenerated up to 10 times via a standard low-pH protocol in as little as two minutes for select applications such as acquisition of replicate data (same ligand/analyte pair) and “bucket”-based screening applications. Regeneration dissociates the GST-containing protein from the factory-immobilized anti-GST antibody, allowing additional analyses. For the highest quality affinity and kinetic results, using a new anti-GST biosensor for each unique capture ligand is recommended.

Materials required

- Octet instrument with Octet software
- Anti-GST biosensors (ForteBio part no. 18-5096 [tray]; 18-5097 [pack]; 18-5098 [case])
- For all Octet instruments: 96-well, black, flat bottom, polypropylene microplate (Greiner Bio-One part no. 655209)

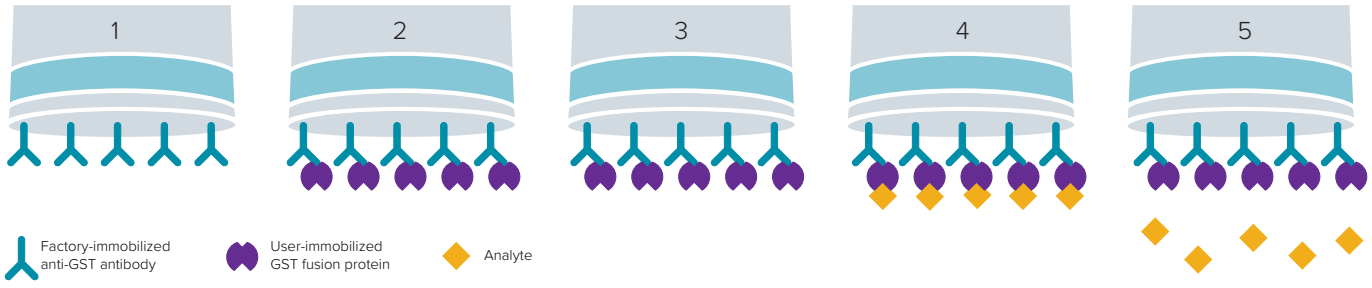

Figure 1: Example workflow for kinetic characterization of the interaction between a GST fusion protein and a target analyte. The assay consists of 5 assay steps. Step 1: equilibration, Step 2: loading (capture) of GST-fusion, Step 3: baseline, Step 4: association kinetics, Step 5: dissociation kinetics.

- 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)
- GST-containing protein for immobilization. The GST-containing protein can be present in either buffer or a complex mixture such as culture supernatant.
- Analyte proteins that interact with GST-fusion protein. The analyte proteins can be dissolved in buffer matrix or a complex mixture such as culture supernatant.
- Kinetics Buffer. The GST biosensor is compatible with a wide range of buffers although 1X Kinetics Buffer is recommended (dilute 10X Kinetics buffer 1:10 with PBS, pH 7.4). Best results are obtained when all matrices are matched as closely as possible.
- Regeneration Buffer. Regeneration Buffer (10 mM glycine, pH 1.7) is required for surface conditioning and regeneration. Further evaluation of the most appropriate pH and optimization may be required.

Tips for optimal performance

- To improve baseline and surface stability, condition the biosensors before the first loading step. Typically, conditioning consists of three 20-second exposures to Regeneration Buffer alternating with 1X Kinetics Buffer.
- Priming the biosensor surface with a binding and regeneration cycle is recommended for the most rigorous kinetic applications. The priming binding cycle consists of capturing GST-containing protein from solution and then regenerating the biosensors back to the original surface with the regeneration protocol. Biosensor priming should follow biosensor conditioning.
- The inter-step correction software processing feature corrects for misalignment between the association and dissociation steps. For the most effective inter-step correction, the baseline and the dissociation steps of an assay cycle should be performed in the same microplate well.
- Typically, the biosensor surface can be regenerated by cycling it between 10 mM glycine pH 1.7 (5 seconds) and 1X Kinetics Buffer (5 seconds) 3 times. Depending on the GST-containing protein being used, the regeneration buffer formulation (buffering element and pH) may require optimization.
- Use of a reference biosensor to correct for drift is recommended. A reference biosensor should be loaded with the GST-containing protein and run with a buffer blank for the association and dissociation steps.
- Equilibrate reagents and samples to room temperature prior to preparation. For frozen samples, thaw and mix thoroughly prior to use.
- 10 minutes of biosensor hydration is required prior to an assay. Hydrating the biosensors in a buffer consistent with the buffer used throughout the assay is recommended.
- Ensure that the Octet instrument is turned on and the lamp is warmed to room temperature 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 and entering the desired temperature. ForteBio recommends assaying at 30°C. Using other temperatures may require modifying the assay times discussed in this protocol.
- The anti-GST biosensor is constructed using elements of the protein streptavidin. When using biotinylated analytes or matrices that contain biotinylated molecules, it is recommended that open biotin binding pockets be blocked by incubating the biosensors in 15 µg/mL of biocytin for 15 minutes.
- Some systems can intermittently display a small “bump” artifact during the transition from the association step to the dissociation step. Increasing the concentration of the BSA carrier protein by utilizing 10X kinetics buffer rather than 1X Kinetics Buffer typically removes this artifact.

Assay protocol

OVERVIEW

1. Prepare assay solutions.
2. Prepare the sample plate.
3. Equilibrate both the hydrated biosensor assembly and the assay plate for 10 minutes on the Octet instrument.
4. Run the assay.
5. Process and analyze the data.
6. Save the results.

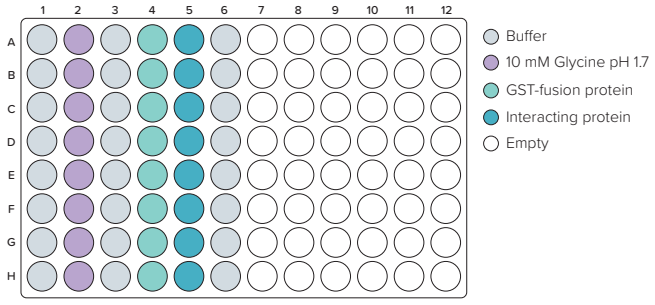

Figure 2: Example plate map for a kinetic assay that includes a pre-conditioning cycle, ligand loading, association, dissociation and a regeneration cycle. An assay step list using the sample plate is described in Table 1. The same sample wells (column 3) are used for the baseline and dissociation steps for optimal use of the inter-step correction processing feature.

Step	Step name	Time (s)	Shake speed	Step type
1	Equilibration	180–600	1000	Custom
2	Conditioning (10 mM Glycine)	20	1000	Custom
3	Neutralization	20	1000	Custom
4	Conditioning (10 mM Glycine)	20	1000	Custom
5	Neutralization	20	1000	Custom
6	Conditioning (10 mM Glycine)	20	1000	Custom
7	Neutralization	20	1000	Custom
8	Equilibration	300–600	1000	Custom
9	Loading	300–600	0–1000	Custom
10	Baseline	180–600	1000	Baseline
11	Association	600–1800	1000	Association
12	Dissociation	600–3600	1000	Dissociation
13	Regeneration (10 mM Glycine)	5	1000	Custom
14	Neutralization	5	1000	Custom
15	Regeneration (10 mM Glycine)	5	1000	Custom
16	Neutralization	5	1000	Custom
17	Regeneration (10 mM Glycine)	5	1000	Custom
18	Neutralization	5	1000	Custom
19	Regeneration (10 mM Glycine)	5	1000	Custom
20	Neutralization	5	1000	Custom
21	Regeneration (10 mM Glycine)	5	1000	Custom
22	Neutralization	5	1000	Custom

Table 1: Example assay steps and associated parameters. After completing step 22, steps 8–22 would be repeated. Step types correspond to Octet software version 5.0 and greater. The same sample wells are used for the baseline and dissociation steps for optimal use of the inter-step correction processing feature.

PREPARE THE SAMPLES AND THE CALIBRATION STANDARDS

Equilibrate reagents and samples to room temperature prior to preparation and mix thoroughly.

1 **GST-containing ligand:** The ligand is the protein that will be immobilized on the biosensor tip surface. GST-containing ligands are typically immobilized at a concentration between 5–25 µg/mL. If the GST-containing ligand is below 5 µg/mL, a longer loading time may be required (60 minutes loading at 1 µg/mL is equivalent to 10 minutes loading at 10 µg/mL). The ligand solution can be recovered from the well after the assay and re-used, if desired. If the ligand is captured from a cell culture supernatant dilution of the supernatant twofold or greater with 1X Kinetics Buffer can potentially increase data quality. If dilution results in a low total concentration of the ligand, the biosensors can be incubated in the supernatant overnight at 4°C to maximize loading of the GST-containing ligand. For tips on optimizing overnight loading, see ForteBio Technical Note 10, *Batch Immobilization of a Biotinylated Ligand onto Streptavidin Biosensors*.

2 **Interacting protein (analyte):** During rigorous kinetic analysis, it is recommended to run a dilution series of at least four concentrations of the analyte protein. The highest concentration should be approximately 10 times the expected K_D. For example, concentrations of 90 nM, 30 nM, 10 nM and 3 nM would be recommended for an analyte with low-nanomolar affinity towards an immobilized ligand. 200 µL/well, 80 µL/well and 40 µL/well of analyte solution are required for 96-well, 384-well, and 384-well tilted-well plates, respectively. The solution can be recovered from the well after the assay and re-used, if desired. For screening assays or qualitative interaction analysis, a single concentration of the interacting protein can be sufficient to characterize the binding.

3 **Conditioning/regeneration solution:** The capture GST-containing ligand and the analyte can be removed from the biosensors by exposing them to 10 mM glycine pH 1.7 followed by a neutralization buffer (typically 1X Kinetics Buffer). 200 µL/ well, 80 µL/well and 40 µL/well of regeneration and neutralization solutions are required for 96-well, 384-well, and 384-well tilted-well plates, respectively. After regeneration, the biosensor can be re-loaded with GST-containing protein for a new interaction analysis. A small loss in binding capacity after a regeneration cycle is expected. Regeneration provides a cost-effective format for generating replicate data for ligand-analyte pairs. For the highest quality kinetic results, using a new biosensor to capture each unique ligand is recommended.

RUNNING THE ASSAY

1. Pipette 200 µL/well of biosensor hydration solution into wells of a 96-well black, flat-bottom microplate corresponding to the number and the positions of biosensors to be used.
2. Insert the hydration plate into the biosensor tray. Align the biosensor rack over the hydration plate and lower the biosensors into the wells, taking care not to scrape or touch the bottom of the biosensors.
3. Transfer 200 µL of each assay reagent into the appropriate wells of a black polypropylene microplate. Place the assay plate on the sample plate stage with well A1 toward the back right corner.
4. Place the biosensor hydration assembly in the Octet instrument on the left stage. Ensure that both the biosensor tray and sample plate are securely in place.
5. Ensure that the Octet instrument and computer are turned on. It is essential that the instrument lamp warms up for at least 40 minutes before running an experiment.
6. Equilibrate the plates in the instrument for 10 minutes prior to starting the experiment. The delay timer can be used to automatically start the assay after 10 minutes (600 seconds).
7. Set up a kinetic assay. For details, see the Octet Data Acquisition User Guide. Table 1 shows an example kinetic assay consisting of a pre-conditioning cycle, ligand loading, association, dissociation and regeneration steps. Figure 2 shows an example plate map for a typical kinetic assay.
8. Run the assay.

PROCESS AND ANALYZE THE DATA

1. Load data into the Octet Data Analysis software.
2. Process the data by specifying methods for reference subtraction, y-axis alignment, inter-step correction and Savitzky-Golay filtering. For details on each processing parameter, refer to the Octet Data Analysis User Guide.
3. Analyze the data by specifying steps for analysis, fitting method (local or global) and window of interest. For details on each analysis parameter, refer to the Octet Data Analysis User Guide.
4. To export the analyzed data, use the Save Report button to generate a Microsoft Excel report. For details on data exporting, refer to the Octet Data Analysis User Guide.

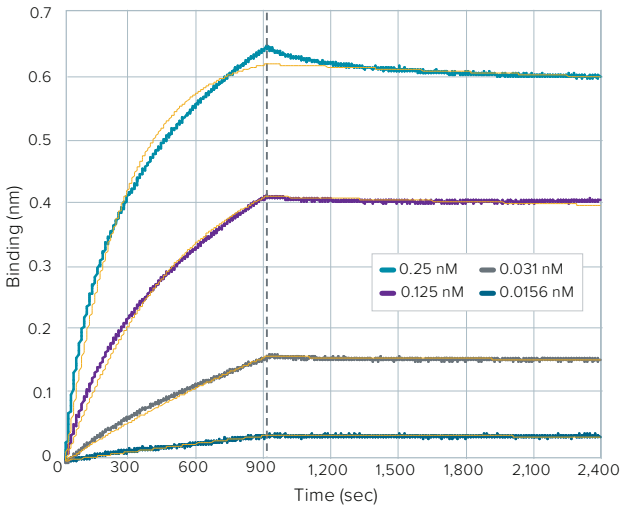

Figure 3: Kinetic analysis of the interaction between GST-Protein A (65 kDa) and an interaction partner Protein B (40 kDa). 10X kinetics buffer was used as the matrix throughout and the assay temperature was 30°C. A single column of biosensors was used to collect three datasets with regeneration using glycine pH 1.7 between cycles (cycles 2 and 3 not shown). Data for cycles 1–3 were processed and curve fit using a 1:1 binding model. The kinetic results for cycles 1–3 are reported in Table 2.

Cycle	k_{on}	k_{off}	k_D
1	$1.5x10^{4}$	$1.9x10^{-5}$	1.2 nM
2	$8.5x10^{3}$	$4.0x10^{-5}$	4.7 nM
3	$6.8x10^{3}$	1.810^{-5}	2.6 nM

Table 2: Kinetic results for the interaction between GST-Protein A and Protein B over three cycles using anti-GST biosensors. Anti-GST biosensors were regenerated using glycine pH 1.7 between cycles.

Representative data

Figure 3 shows kinetic analysis of the interaction between GST-Protein A (65 kDa) and an interaction partner Protein B (40 kDa). Both proteins were heterologously expressed in *E. coli* and purified. Anti-GST biosensors were hydrated for 10 minutes in 10X Kinetics Buffer prior to analysis. Assays steps included: preconditioning, (3 cycles of alternating 5 dips in glycine pH 1.7 and 10X kinetics buffer), 5 minutes of equilibration, 5 minutes of GST-Protein A loading (5 µg/mL), 5 minutes of baseline stabilization, 15 minutes of GST-Protein A : Protein B association and 25 minutes of GST-Protein A : Protein B dissociation. Analyte concentrations were .016, .013, .125 and .25 nM. 10X Kinetics Buffer was used as the matrix throughout and the assay temperature was 30°C. A single column of biosensors was used to collect three datasets with regeneration using glycine pH 1.7 between cycles (cycles 2 and 3, not shown). Data for cycles 1–3 were processed and curve fit using a 1:1 binding model. The kinetic results for cycles 1–3 are reported in Table 2.

FORTÉBIO

www.fortebio.com

ForteBio
47661 Fremont Boulevard
Fremont, CA 94538
888.OCTET-75 or 650.322.1360
fortebio.info@moldev.com

ForteBio Analytics (Shanghai) Co., Ltd.
No. 88 Shang Ke Road
Zhangjiang Hi-tech Park
Shanghai, China 201210
salesops.china@moldev.com

Molecular Devices (UK) Ltd.
660-665 Eskdale
Winnersh Triangle
Wokingham, Berkshire
RG41 5TS, United Kingdom
+44 118 944 8000
uk@moldev.com

Molecular Devices (Germany) GmbH
Bismarckring 39
88400 Biberach an der Riss
Germany
+ 00800 665 32860

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