

GST Sensors

Tech Guide



Immobilization method:	Capture
Ligand requirements:	GST tag
Ligand orientation:	Oriented via tag
Recommended coupling kit:	GST Reagent Kit

Sensor storage buffer	PBS
Recommended storage	4°C
Shelf life	6 months

Overview

The GST Sensors have a uniform functional coating of glutathione (GSH) for capture of ligands containing Glutathione-S-Transferase (GST) tags. GST-tags are commonly used for protein purification and are a convenient method for immobilization. This sensor surface will provide oriented capture of the ligand via the GST tag (Figure 1). The GST Reagent Kit provides a GST priming solution to increase the binding activity of the sensor surface.

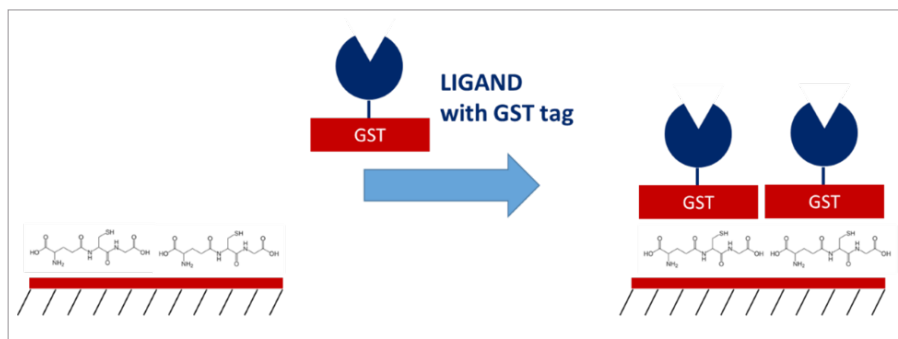


Figure 1. Immobilization of GST-tagged ligand onto a GST Sensor.

Materials and Reagents Required for Coupling:

- GST Sensor
- GST Reagent Kit
 - GST Priming Solution
- 10 mM NaOH

Injection Volumes

Minimum recommended injection volumes for a 100 μ L sample loop:

OpenSPR Rev 4	150 μ L
OpenSPR-XT Rev 4	200 μ L
OpenSPR Rev 3	200 μ L
OpenSPR-XT Rev3	300 μ L

Buffer Conditions

Conditions to avoid:

- Buffers containing free glutathione (GSH)

Running buffers containing GSH may reduce the effective binding of the GST tag to the sensor surface as it will compete.

Ligand Removal

GST-tagged ligands can often be removed from the GST sensor by adding the regeneration solution containing reduced glutathione (GSH). Ligand removal is only necessary if efficient regeneration of the ligand (dissociation of analyte) is not possible without affecting the structural integrity of the ligand.

Referencing

For the 2-Channel OpenSPR, it is recommended to immobilize the ligand in channel 2 only and use a blocked sensor surface (with free GST protein or an inactive GST-tagged protein only) in channel 1 as the reference channel. For a non-specific binding experiment using the 1-Channel OpenSPR, it is recommended to prepare a blocked sensor surface (with free GST protein or an inactive GST-tagged protein only) as a negative control. This will block the GSH groups with a protein of similar size to your ligand, simulating the open sites on the ligand-bound sensor. Next, inject an analyte at the highest concentration to be used for the experiment.

Coupling Procedure

1. Surface Conditioning

Perform an injection of 10 mM NaOH to clean the sensor surface.

CH	Flow Rate
1+2	150 μ L/min

2. Surface Activation

Prepare fresh GST Priming solution according to the GST Reagent Kit Tech Guide. Perform an injection of the prepared GST Priming Solution to activate the GST Sensor surface. (5-minute interaction time)

CH	Flow Rate
1+2	20 μ L/min

3. Ligand Immobilization

Dilute the GST-tagged ligand to be immobilized in running buffer to a concentration of 10-100 μ g/mL. Inject the ligand solution into the instrument. (5-minute interaction time)

CH	Flow Rate
2	20 μ L/min

Evaluation

The amount of ligand binding is calculated by comparing the signal after the GST Priming Solution injection to the signal after the ligand immobilization step. In the example shown in *Figure 2*, it is approximately 650 RU. Ensure this meets your minimum ligand immobilization target.

If your immobilization target is not reached, repeat another ligand immobilization injection, or consider optimization of this step.

4. Blocking

Inject GST protein or an inactive GST-tagged protein to block the open sites on the sensor. (5-minute interaction time)

CH	Flow Rate
1+2	20 μ L/min

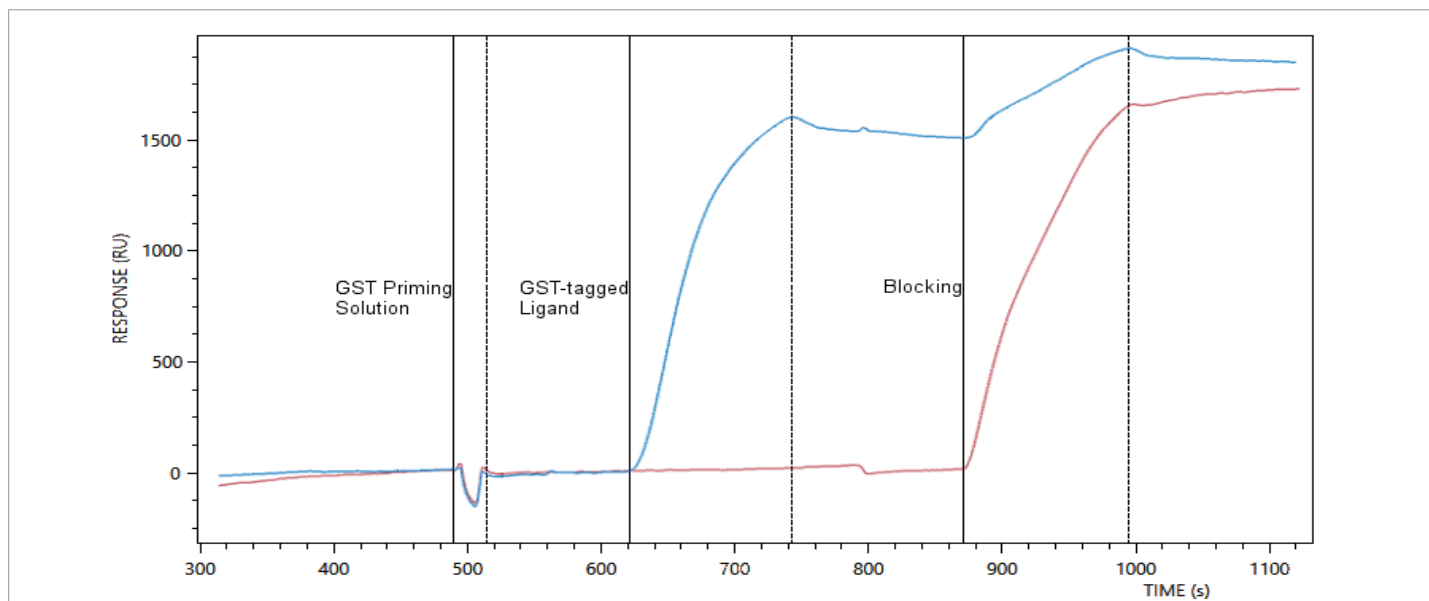


Figure 2. Example of GST Sensor GST-tagged protein immobilization on the 2-Channel OpenSPR system (red: Channel 1, blue: Channel 2).