GST Capture Kit

Instructions for Use

Product description

Product code:	BR100223
Contents:	• Anti-GST antibody, 0.6 mg/ml in 0.15 M NaCl, 100 µL • Recombinant GST (*Schistosoma japonicum*, molecular weight 26 kDa), 0.2 mg/ml in HBS-EP buffer (10 mM HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, and 0.005% Surfactant P20), 100 µL • Immobilization buffer, 10 mM Sodium acetate pH 5.0, 5 mL • Regeneration solution, 10 mM Glycine-HCl pH 2.1, 70 mL
Storage:	2°C to 8°C
Kit capacity:	The kit contains reagents sufficient for 20 immobilizations and up to 600 regenerations.
Safety:	For use and handling of the product in a safe way, refer to the Safety Data Sheet.

Note: *For research use only.*

Intended use

GST Capture Kit is intended for site-directed capture of glutathione-S transferase (GST) fusion proteins for biomolecular interaction analysis using Biacore™ systems.

Anti-GST Antibody is suitable for immobilization on sensor chip surfaces using Amine Coupling Kit and the included immobilization buffer. Regeneration solution is used for regeneration of the surface by removal of the captured fusion protein and any associated molecules.

Antibody information

Anti-GST antibody is a polyclonal goat antibody recognizing GST.

Required materials

See the list below for additional required materials (available from Cytiva).

- Sensor chip (Sensor Chip CM5, CM4, or CM3)
- Amine Coupling Kit
- Running buffer (e.g., HBS-EP+. HBS-P+, HBS-N, PBS-P+, or PBS)

Note: *Refer to Instructions for Use for the respective sensor chip.*

Recommended immobilization conditions

Antibody preparation

Centrifuge and mix Anti-GST antibody before use. Dilute the antibody to 30 µg/mL in immobilization buffer (e.g., 5 µL Anti-GST antibody + 95 µL immobilization buffer).

Active and references surfaces

Immobilize the active and reference surfaces using the same settings for both flow cells.

Perform two identical immobilizations in adjacent flow cells.

For use in Biacore 4000, perform the immobilization in spots 1 + 2 and/or 5 + 4 in one injection by ticking the ***Immobilize for capture*** box in the immobilization wizard.

Note: *Do not use an unmodified surface as a reference.*

Immobilization settings

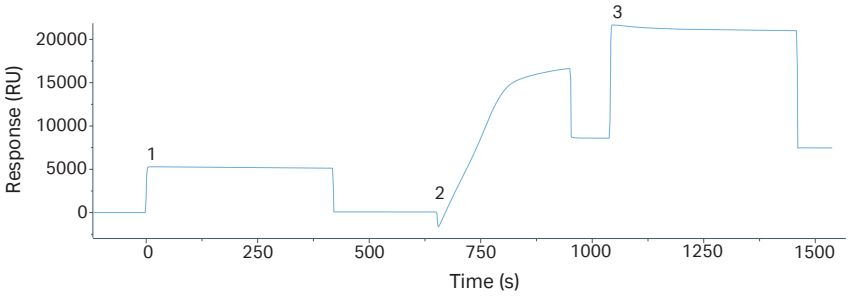
Reagents for immobilization are provided in the Amine Coupling Kit.

Perform immobilization at 25°C using a flow rate of 5 to 10 µL/min in systems where flow rate can be adjusted. The immobilization procedure is shown in the table below.

Procedure step	Injection	Recommended conditions
Activation	EDC/NHS	• All Biacore systems, except Biacore 4000: 7 minutes • Biacore 4000: 10 minutes
Immobilization	Anti-GST antibody	5 minutes
Deactivation	Ethanolamine	7 minutes

This procedure typically results in immobilization levels of 5000 RU or more on Sensor Chip CM5. At these levels, the exact amount of immobilized anti-GST antibody is normally not critical for capturing GST fusion proteins. The immobilization level can be adjusted if necessary by adjusting the contact time or concentration of anti-GST antibody.

The sensorgram below shows a typical immobilization sequence for Anti-GST antibody on Sensor Chip CM5. The numbers indicate the start of injections of EDC/NHS (1), Anti-GST antibody (2), and Ethanolamine (3).



Blocking high affinity sites

The polyclonal Anti-GST antibody carries a minor fraction of high affinity sites that are difficult to regenerate. To avoid capture of GST-tagged ligand on these sites, block the high affinity sites with recombinant GST after immobilization by running 1 to 3 cycles using a 3-minute injection of recombinant GST at 5 µg/mL in running buffer followed by regeneration as described in *Regeneration injection, on page 4*.

Recommended running conditions

Analysis temperature

GST Capture Kit is designed for use at 25°C. Other temperatures might work but have not been tested.

Start-up cycles

For best assay performance, run at least one start-up cycle using identical settings as for the analysis cycles, including capture, analyte, and regeneration injection. Replace the analyte with running buffer.

Capture injection

Conditions for ligand capture will depend on the concentration and binding characteristics of the ligand and the purpose of the experiment. Typical conditions are injection of ligand at 2 to 10 µg/ml with a contact time of 3 minutes.

Analyte injection

Use analyte injection conditions appropriate for the assay purpose.

Regeneration injection

Regeneration removes the ligand and any binding partner, leaving anti-GST antibody on the surface. For most GST-fusion proteins, regeneration with a 2-minute injection of the regeneration solution included in the kit is suitable.

For proteins where these conditions do not give adequate regeneration, an additional 1-minute injection of one of the following solutions can be tested:

- 10 mM NaOH
- 0.1% SDS
- 0.1% trifluoroacetic acid
- 3 M $MgCl_2$
- 30% ethylene glycol in 10 mM glycine-HCl, pH 2.0

For more information on running conditions for different applications, guides, lab protocols, and free eLearnings, visit *cytiva.com/biacore*.

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