GE Healthcare
Life Sciences

Instruction 22-0522-19 AG **Biacore™**

GST Capture Kit

Product description

Order code:	BR-1002-23
Contents:	• Anti-GST antibody: immunosorbent-purified polyclonal goat 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, 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 to 8°C
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, please refer to the Safety Data Sheet.

Note: *For in vitro use only.*

Intended use

GST Capture Kit is intended for site-directed capture of GST (glutathione-S transferase) fusion proteins for biomolecular interaction analysis using Biacore systems. Anti-GST antibody is suitable for immobilization on carboxyl-derivatized sensor chips 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.

Immobilization procedure

Required materials

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

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

Preparation

Dilute Anti-GST antibody to 30 µg/ml in immobilization buffer (5 µl anti-GST antibody + 95 µl immobilization buffer).

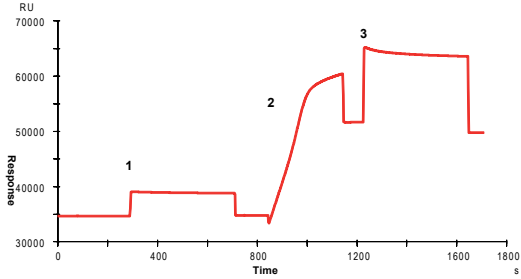
Immobilization settings

Perform immobilization at 25°C using a flow rate of 5 to 10 µl/min (in systems where flow rate can be adjusted). Reagents for immobilization are provided in Amine Coupling Kit.

Step	Injection	Conditions
Activation	EDC/NHS	• Biacore 4000 and Biacore A100: 10 minutes • Other Biacore instruments: 7 minutes
Immobilization	Anti-GST antibody	• Biacore 3000: 4 minutes • Other Biacore instruments: 5 minutes
Deactivation	Ethanolamine	7 minutes

This procedure should result in immobilization levels of 7500 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 may 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 below (see *Regeneration injection, on page 5*).

Reference surface

The reference surface should be prepared in the same way as the active surface, that is the reference surface should be immobilized using the same settings as the active surface.

For use in Biacore 4000 and Biacore A100, 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.

For use in Biacore T200 and other instruments, perform two identical immobilizations in adjacent flow cells.

Note: *It is not recommended to use an unmodified surface as a reference.*

Run conditions

Running buffer

Commonly used buffers for analysis: HBS-EP+, HBS-EP, HBS-P+, HBS-P, HBS-N, PBS-P+, PBS (available from GE Healthcare).

Start-up cycles

For best assay performance, run at least one start-up cycle using identical settings as for the analysis cycles, including GST-tagged ligand and buffer instead of analyte.

Ligand 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.

Sample injection

Use sample injection conditions appropriate to 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 may 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

Reference surface

In systems that support injection over the reference surface before capturing the ligand on the active surface, use Recombinant GST from the kit at a molar concentration similar to the concentration used for the ligand.

For local office contact information, visit
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