GST-Trap®_A Kit for Immunoprecipitation of GST-Fusion Proteins from mammalian cell extract

Only for research applications, not for diagnostic or therapeutic use.

Introduction

For biochemical analyses including mass spectrometry and enzyme activity measurements Glutathione S-transferase (GST)-fusion proteins and their interacting factors can be isolated fast and efficiently in one step by immunoprecipitation using the GST-Trap. GST-Trap_A contains a small GST-binding protein covalently coupled to the surface of agarose beads. It enables the purification of any protein of interest fused to GST.

Specificity

GST of *Schistosoma japonicum*

Content

Reagent	Code	Quantity
GST-Trap®_A kit	stak-20	20 reactions (0.5 ml resin)
Lysis buffer (CoIP)		30 ml
RIPA buffer		30 ml
5x Wash / Dilution buffer		2 x 10 ml
Elution buffer		3 x 1 ml

Note: Add 40 ml H_2O to 5x Wash/ Dilution buffer before use. It is 5 times concentrated!!
Note: 0,09 Na-Azide is added to our buffers as an antiseptic and antifungal agent.
Note: For other cell types like yeast, plants, drosophila, etc. please use your equivalent cell lysis buffer.

Bead properties

Bead size: ~ 90 µm (cross-linked 4% agarose beads)
Storage buffer: 20% EtOH
Binding capacity: 10 µl GST-Trap®_A slurry binds 3-4 µg of GST

Stability and Storage

Shipped at ambient temperature. Upon receipt store at +4°C.
Stable for 1 year. Do not freeze.

Required solutions

Buffer composition (as provided in the kit)

Buffer	Composition
Lysis buffer (CoIP)	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA; 0.5% NP-40, 0.09% Na-Azide
RIPA buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0,5 mM EDTA; 0,1% SDS; 1% Triton X-100; 1% Deoxycholate, 0.09% Na-Azide
Dilution/Wash buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA, 0.018% Na-Azide
Elution buffer	200 mM glycine pH 2.5

Related products

GST Toolbox	Code
GST-Trap® protein	st-250
GST-Trap®_A	sta-20; sta-100; sta-200; sta-400
Blocked agarose beads	bab-20
GST antibody	6g9
Spin columns	sct-10; sct-20; sct-50

Support

Please refer to our FAQ section at *www.chromotek.com* or contact *support@chromotek.com*

Protocol for Immunoprecipitation of GST-Fusion Proteins using GST-Trap®_A

Harvest cells

For one immunoprecipitation reaction the use of ~10^6 - 10^7 mammalian cells (approx. one 10-cm dish) expressing a GST-tagged protein of interest is recommended. To harvest adherent cells, aspirate growth medium, add 1 ml ice-cold PBS to cells and scrape cells from dish. Transfer cells to a pre-cooled tube, spin at 500 g for 3 min at +4°C and discard supernatant. Wash cell pellet twice with ice-cold PBS, gently resuspending the cells. After washing:

Lyse cells

1. Resuspend cell pellet in 200 µl ice-cold lysis buffer by pipetting or using a syringe.

   note: Supplement lysis buffer with protease inhibitors and 1 mM PMSF (not included).

   optional for nuclear/chromatin proteins: Use RIPA buffer supplemented with 1 mg/ml DNase, 2.5 mM $MgCl_2$, protease inhibitors and 1 mM PMSF (not included).
2. Place the tube on ice for 30 min with extensively pipetting every 10 min.
3. Centrifuge cell lysate at 20.000x g for 10 min at +4°C. Transfer lysate to a pre-cooled tube. Add 300 µl dilution buffer to lysate. Discard pellet.

   note: At this point cell lysate may be put at -80°C for long-term storage.

   optional: Add 1 mM PMSF and protease inhibitors (not included) to dilution buffer.

Equilibrate beads

4. Vortex GST-Trap®_A beads and pipette 25 µl bead slurry into 500 µl ice-cold dilution buffer. Centrifuge at 2.500x g for 2 min at +4°C. Discard supernatant and repeat wash twice.

Bind proteins

5. Add diluted lysate (step 3) to equilibrated GST-Trap®_A beads (step 4). If required, save 50 µl of diluted lysate for immunoblot analysis. Tumble end-over-end for 1 hour at 4°C.
6. Centrifuge at 2.500x g for 2 min at +4°C. If required, save 50 µl supernatant for immunoblot analysis. Discard remaining supernatant.

Wash beads

7. Resuspend GST-Trap®_A beads in 500 µl ice-cold dilution buffer. Centrifuge at 2.500x g for 2 min at +4°C. Discard supernatant and repeat wash twice.

   optional: Increase salt concentration in the second washing step up to 500 mM.

Elute proteins

8. Resuspend GST-Trap®_A beads in 100 µl 2x SDS-sample buffer.
9. Boil resuspended GST-Trap®_A beads for 10 min at 95°C to dissociate immunocomplexes from GST-Trap®_A beads. GST-Trap®_A beads can be collected by centrifugation at 2.500x g for 2 min at 4°C and SDS-PAGE is performed with the supernatant.
10. *optional instead of steps 8 and 9: elute bound proteins by adding 50 µl 0.2 M glycine pH 2.5 (incubation time: 30 sec under constant mixing) followed by centrifugation. Transfer the supernatant to a new tube and add 5 µl 1M Tris base pH 10.4 for neutralization. To increase elution efficiency this step can be repeated.*