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

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

Introduction

Green fluorescent proteins (GFPs) and variants thereof are widely used to study protein localization and dynamics. For biochemical analysis including mass spectrometry and enzyme activity measurements these GFP-fusion proteins and their interacting factors can be isolated fast and efficiently by immunoprecipitation using the GFP-Trap®. GFP-Trap® utilizes small recombinant alpaca antibody fragments covalently coupled to the surface of agarose beads.

Specificity

tested on eGFP, GFP, tagGFP, YFP, CFP, Venus, Citrine, AcGFP

Content

Reagent	Code	Quantity
GFP-Trap®_A kit	gtak-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 GFP-Trap®_A slurry binds 12 µg of recombinant GFP

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

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.

Related products

GFP Toolbox	Code
GFP-Trap® protein	gt-250
GFP-Trap®_A	gta-20; gta-100; gta-200; gta-400
GFP-multiTrap	gtp-96; gtp-480
Blocked agarose beads	bab-20
GFP antibody	3h9
GFP-Booster_Atto488	gba-488
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 GFP-Fusion Proteins using GFP-Trap®_A

Harvest cells

For one immunoprecipitation reaction the use of ~10^6 - 10^7 mammalian cells (approx. one 10-cm dish) expressing a GFP-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 GFP-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 GFP-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 GFP-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 GFP-Trap®_A beads in 100 µl 2x SDS-sample buffer.
9. Boil resuspended GFP-Trap®_A beads for 10 min at 95°C to dissociate immunocomplexes from GFP-Trap®_A beads. GFP-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.*