GFP-Trap®_A for Immunoprecipitation of GFP-Fusion Proteins

For the immunoprecipitation of GFP-fusion-proteins from cellular extracts.
Only for research applications, not for diagnostic or therapeutic use

Introduction

Green fluorescent proteins (GFP) and variants thereof are widely used to study protein localization and dynamics. For biochemical analyses including mass spectrometry and enzyme activity measurements these GFP-fusion proteins and their interacting factors can be isolated fast and efficiently in one step by immunoprecipitation using the GFP-Trap®. GFP-Trap®_A contains a small GFP-binding protein covalently coupled to the surface of agarose beads. It enables the purification of any protein of interest fused to GFP, eGFP, YFP, CFP or Venus (for a complete list of recognized GFP variants, please visit the FAQ section at www.chromotek.com).

Content

Reagent	Code	Quantity
GFP-Trap®_A	gta-20	20 reactions (0.5 ml slurry)
GFP-Trap®_A	gta-100	100 reactions (2.5 ml slurry)
GFP-Trap®_A	gta-200	200 reactions (5 ml slurry)
GFP-Trap®_A	gta-400	400 reactions (10 ml slurry)

Bead properties

Bead size: ~ 90 µm
Storage buffer: 20% EtOH
Binding capacity: 10 µl GFP-Trap®_A slurry binds 2.5 – 3 µg of GFP

Stability and Storage

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

Required solutions

Suggested buffer composition

Buffer	Composition
Lysis buffer (CoIP)	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA; 0.5% NP-40
10x RIPA buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 5 mM EDTA; 0.1% SDS; 1% Triton X-100; 1% Deoxycholate
Dilution buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA
Wash buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA
Elution buffer	200 mM glycine pH 2.5

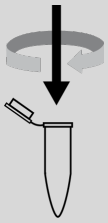
Related products

GFP Toolbox	code
GFP-Trap®_A Kit	gtak-20
GFP-Trap®_M	gtm-20; gtm-100; gtm-200; gtm-400
GFP-Trap®_M Kit	gtmk-20
GFP-multiTrap	gtp-96; gtp-480
Blocked agarose beads	bab-20
Blocked magnetic beads	bmp-20
GFP antibody	3h9
GFP-Booster_Atto488	gba-488

Protocol

Harvest.

Before you start: Add 1ml PBS to your cells and scrape them off the petri dish. Transfer to precooled tube, spin 3 min at 500 g and discard supernatant. **Wash cell pellet twice** with ice cold PBS, briefly resuspending the cells.

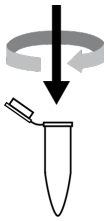


Lyse cells

1. For one immunoprecipitation reaction resuspend cell pellet (~10^7 mammalian cells) in 200 µl lysis buffer by pipetting (or using a syringe).

   optional: add 1 mM PMSF and Protease inhibitor cocktail (not included) to lysis buffer
   optional for nuclear/chromatin proteins: add 1 mg/ml DNase and 2.5 mM $MgCl_2$ (not included) to lysis buffer

2. Place the tube on ice for 30 min with extensively pipetting every 10 min.
3. Spin cell lysate at 20.000x g for 5 -10 minutes at 4°C.



Bind GFP-fusion protein

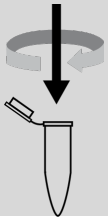
4. Transfer supernatant to a pre-cooled tube. Adjust volume with dilution buffer to 500 µl – 1000 µl. Discard pellet.

   optional: add 1 mM PMSF and Protease inhibitor cocktail (not included) to dilution buffer
   note: the cell lysate can be frozen at this point for long-term storage at -80°C

   For immunoblot analysis dilute 50 µl cell lysate with 50 µl 2x SDS-sample buffer (→ refer to as input).

5. Equilibrate GFP-Trap®_A beads in dilution buffer. Resuspend 20 - 30 µl bead slurry in 500 µl ice cold dilution buffer and spin down at 2.500x g for 2 minutes at 4°C. Discard supernatant and wash beads 2 more times with 500 µl ice cold dilution buffer.
6. Add cell lysate to equilibrated GFP-Trap®_A beads and incubate the GFP-Trap®_A beads with the cell lysate under constant mixing for 10 min – 2 h at room temperature or 4°C.

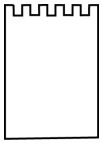
   note: during incubation of protein sample with the GFP-Trap®_A the final concentration of detergents should not exceed 0.2% to avoid unspecific binding to the matrix



Wash (3x)

7. Spin tube at 2.500x g for 2 minutes at 4°C. For western blot analysis dilute 50 µl supernatant with 50 µl 2x SDS-sample buffer (→ refer to as non-bound). Discard remaining supernatant.
8. Wash beads three times with 500 µl ice cold wash buffer. After the last wash step, transfer beads to new tube.

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



Load gel, or directly use in downstream application

9. Resuspend GFP-Trap®_A beads in 100 µl 2x SDS-Sample buffer or go to step 11.
10. Boil resuspended beads for 10 minutes at 95°C to dissociate the immunocomplexes from the beads. The beads can be collected by centrifugation at 2.500x g for 2 minutes at 4°C and SDS-PAGE is performed with the supernatant (→ refer to as bound).
11. *optional: 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 fresh cup and add 5 µl 1M Tris base (pH 10.4) for neutralization. To increase elution efficiency this step can be repeated.*

Support

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

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