GFP-Trap®_A for Immunoprecipitation of GFP-Fusion Proteins (gta)

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

1. Introduction

Green fluorescent proteins (GFP) and variants thereof are widely used to study protein localization and dynamics. For biochemical analyses including mass spectroscopy and enzyme activity measurements these GFP-fusion proteins and their interacting factors can be isolated fast and efficiently (one step) via Immunoprecipitation using the GFP-Trap®. The GFP-Trap®_A enables purification of any protein of interest fused to GFP.

2. Content

GFP-Trap®_A (bead size: ~ 80 µm) in 20% EtOH

3. Stability and Storage

Store material at 2-8°C, do not freeze.

4. Protocol

1. For one immunoprecipitation reaction resuspend cell pellet (~10^7 cells) in 200 µl lysis buffer by pipetting (or using a syringe)
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
4. Transfer supernatant to a precooled tube. Adjust volume with dilution buffer to 500 µl – 1000 µl. Discard pellet

   The cell lysate can be frozen at this point for long-term storage at minus 80°C. Discard pellet

   For immunoblot analysis dilute 50 µl cell lysate with 50 µl 4x SDS-sample buffer (-> refer as input)
5. Equilibrate GFP-Trap® beads in dilution buffer. Resuspend 20 - 30 µl Beads Slurry in 500 µl ice cold dilution buffer and spin down at 2700x g for 2 minutes at 4°C. Discard supernatant and wash binder two more times with 500 µl ice cold dilution buffer.
6. Add cell lysate to equilibrated GFP-Trap®_A beads
7. Incubate with gentle end-over-end mixing for 10 min – 2 h at room temperature or 4°C
8. Spin tube at 2000x g for 2 minutes at 4°C
9. For western blot analysis dilute 50 µl supernatant with 50 µl 4x SDS-sample buffer (-> refer as non-bound)
10. Discard remaining supernatant
11. Wash pellet two times with 500 µl ice cold dilution buffer

    (optional: increase salt concentration in the second washing step up to 500 mM)
12. Resuspend GFP-Trap®_A beads in 100 µl 2x SDS-Sample buffer
13. Boil resuspended beads for 10 minutes at 95°C to dissociate the immunocomplexes from the beads. The beads can be collected by centrifugation at 2700x g for 2 minutes at 4°C and SDS-PAGE is performed with the supernatant. (-> refer as bound)
14. (optional) elute bound proteins by adding 50 µl 0.1 M glycine pH 2.5 (incubation time: 30 sec – 2 min) followed by neutralisation with 5 µl 1M Tris-base

Suggested Buffers (as tested in our laboratory)

Lysis-buffer (native):
10 mM Tris/Cl, pH 7.5
150 mM NaCl
0.5 mM EDTA
0.5% NP40
1 mM PMSF freshly added (optional)
1x mammalian Protease Inhibitor Cocktail (e.g. Serva®) freshly added

(optional for nuclear proteins / chromatin proteins:
DNaseI final conc. 1 µg/µl
2.5 mM $MgCl_2$)

Dilution-buffer
10 mM Tris/Cl, pH 7.5
150 mM NaCl
0.5 mM EDTA
1 mM PMSF freshly added (optional)
1x Protease Inhibitor Cocktail (e.g. Serva) freshly added

Wash-buffer
10 mM Tris/Cl pH 7.5
150 - 500 mM NaCl
0.5 mM EDTA
1 mM PMSF freshly added (optional)
1x Protease Inhibitor Cocktail (e.g. Serva®) freshly added

RIPA-Buffer (for cell lysis):
10 mM Tris/Cl, pH 7.5
150 mM NaCl
0.1% SDS
1% TX100
1% Deoxycholate
5 mM EDTA
1 mM PMSF freshly added (optional)
1x Protease Inhibitor Cocktail (e.g. Serva®) freshly added