

# GFP-Trap<sup>®</sup>\_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<sup>®</sup>. GFP-Trap<sup>®</sup> 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 <sup>®</sup> _A kit	gtak-20	20 reactions (0.5 ml resin)
Lysis buffer (CoIP)		30 ml
RIPA buffer		30 ml
5x Wash / Dilution buffer		20 ml
Elution buffer		3 ml

## Bead properties

Bead size: ~ 90 µm (cross-linked 4% agarose beads)  
Storage buffer: 20% EtOH  
Binding capacity: 10 µl GFP-Trap<sup>®</sup>\_A slurry binds 3-4 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

### 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
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
Dilution/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 <sup>®</sup> protein	gt-250
GFP-Trap <sup>®</sup> _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

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## Protocol for Immunoprecipitation of GFP-Fusion Proteins using GFP-Trap®\_A

### Harvest cells

For one immunoprecipitation reaction the use of  $\sim 10^6$  -  $10^7$  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  $\mu$ l 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<sub>2</sub>, 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  $\mu$ l 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  $\mu$ l bead slurry into 500  $\mu$ l 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  $\mu$ l 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  $\mu$ l supernatant for immunoblot analysis. Discard remaining supernatant.

### Wash beads

7. Resuspend GFP-Trap®\_A beads in 500  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l 1M Tris base pH 10.4 for neutralization. To increase elution efficiency this step can be repeated.*

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