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TurboGFP-Trap_MA for Immunoprecipitation of TurboGFP-Fusion Proteins from mammalian cell extract

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

Introduction

Fluorescent proteins are widely used to study protein function, localization and dynamics in cells. TurboGFP is a bright dimeric green fluorescent protein derived from CopGFP from the copepod *Pontellina plumata*. Copepod TurboGFP is evolutionarily distant from jellyfish-derived fluorescent proteins such as EGFP. Turbo-GFP shares only ~21 % sequence identity with the commonly used GFP variants. For biochemical analysis including mass spectrometry and enzyme activity measurements, TurboGFP-fusion proteins and their interacting factors can be isolated fast and efficiently by immunoprecipitation using the TurboGFP-Trap. TurboGFP-Trap_MA utilizes small recombinant alpaca antibody fragments covalently coupled to the surface of magnetic agarose beads.

Specificity

TurboGFP, CopGFP

Content

Reagent	Code	Quantity
TurboGFP-Trap_MA	tbtma-10	10 reactions (0.25 ml slurry)
TurboGFP-Trap_MA	tbtma-20	20 reactions (0.5 ml slurry)
TurboGFP-Trap_MA	tbtma-100	100 reactions (2.5 ml slurry)
TurboGFP-Trap_MA	tbtma-200	200 reactions (5 ml slurry)
TurboGFP-Trap_MA	tbtma-400	400 reactions (10 ml slurry)

Bead properties

Bead size: ~ 40 µm
Storage buffer: 20% EtOH
Binding capacity: 10 µl TurboGFP-Trap_MA slurry binds 3-4 µg of recombinant TurboGFP

Stability and Storage

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

Required solutions

Suggested buffer compositions for mammalian cells

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 2.5 100 mM citric acid pH 3.0
2 x SDS-sample buffer	120 mM Tris/Cl pH 6.8; 20% glycerol; 4% SDS, 0.04% bromophenol blue; 10% β-mercaptoethanol

Note

In case you want to apply harsh washing conditions, you can add the following substances to the wash buffer (not the dilution buffer):

Substance	Concentration
NaCl	2 M
Nonidet P40 Substitute	2%
Triton X-100	1%
SDS	0.2%
DTT	10 mM
Urea	6 M
Tested up to specified value. Higher concentrations may be compatible.	

Related products

TurboGFP Toolbox	Code
TurboGFP-Binding Protein	tbt-250
TurboGFP-Trap_MA Kit	tbtmak-20
Binding control magnetic agarose beads	bmab-20

Support

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

Protocol for Immunoprecipitation of TurboGFP-Fusion Proteins using TurboGFP-Trap_MA

Harvest cells

For one immunoprecipitation reaction the use of ~10^6 - 10^7 mammalian cells (approx. one 10-cm dish) expressing a TurboGFP-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.

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 inhibitor cocktail and PMSF (f.c. 1 mM) (not included).

   optional for nuclear proteins: Resuspend cell pellet in 200 µl ice-cold RIPA buffer supplemented with DNaseI (f.c. 75-150 U/ml), $MgCl_2$ (f.c. 2.5 mM), protease inhibitor cocktail and PMSF (f.c. 1 mM) (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 supplemented with PMSF (f.c. 1 mM) and protease inhibitor cocktail (not included) to lysate. Discard pellet.

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

Equilibrate beads

4. Resuspend TurboGFP-Trap_MA beads and pipette 25 µl bead slurry into 500 µl ice-cold Dilution buffer. Magnetically separate beads until supernatant is clear. Discard supernatant and repeat wash twice.

Bind proteins

5. Add diluted lysate (step 3) to equilibrated TurboGFP-Trap_MA 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. Magnetically separate beads until supernatant is clear. If required, save 50 µl supernatant for immunoblot analysis. Discard remaining supernatant.

Wash beads

7. Resuspend TurboGFP-Trap_MA beads in 500 µl ice-cold Wash buffer. Magnetically separate beads until supernatant is clear. Discard supernatant and repeat wash twice.

Elute proteins

Elution with SDS-sample Buffer (Laemmli)

- Resuspend beads in 80 µl 2x SDS-sample buffer.
- Boil beads for 5 min at 95°C to dissociate immunocomplexes from beads.
- Magnetically separate beads. Collect supernatant and analyze samples by SDS-PAGE and Western blot.

Elution with Elution Buffer (low pH)

- Resuspend beads in 50–100 µl Elution buffer and permanently pipet up and down for 30-60 seconds at room temperature.
- Magnetically separate beads and transfer supernatant to a new tube.
- Immediately neutralize solution with 5-10 µl 1 M Tris pH 10.4.
- To increase elution efficiency, steps a-d may be repeated.