RFP-Trap®_MA for Immunoprecipitation of RFP-Fusion Proteins from mammalian cell extract

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

Introduction

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

Specificity

tested on RFP, mCherry, mOrange, mPlum, tagRFP

Content

Reagent	Code	Quantity
RFP-Trap®_MA	rtma-20	20 reactions (0.5 ml slurry)
RFP-Trap®_MA	rtma-100	100 reactions (2.5 ml slurry)
RFP-Trap®_MA	rtma-200	200 reactions (5 ml slurry)
RFP-Trap®_MA	rtma-400	400 reactions (10 ml slurry)

Bead properties

Bead size: ~ 40 µm
Storage buffer: 20% EtOH
Binding capacity: 10 µl RFP-Trap®_MA slurry binds 8 µg of mCherry

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
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
Glycine-elution buffer	200 mM glycine pH 2.5
2 x SDS-sample buffer	120 mM Tris/Cl pH 6.8; 20% glycerol; 4% SDS, 0.04% bromophenol blue; 10% β-mercaptoethanol

Related products

RFP Toolbox	Code
RFP-Trap® protein	rt-250
RFP-Trap®_MA Kit	rtmak-20
Blocked magnetic agarose beads	bmab-20
RFP antibody 3F5 (mouse)	3f5
RFP antibody 5F8 (rat)	5f8
RFP-Booster (ATTO 488, ATTO 594, ATTO 700)	rba-488, rba-594, rba-700
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 RFP-Fusion Proteins using RFP-Trap®_MA

Harvest cells

For one immunoprecipitation reaction the use of ~10^6 - 10^7 mammalian cells (approx. one 10-cm dish) expressing a RFP-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 supernatant to a pre-cooled tube. Add 300 µl dilution buffer to supernatant. 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 RFP-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 RFP-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 RFP-Trap®_MA beads in 500 µl dilution buffer. Magnetically separate beads until supernatant is clear. Discard supernatant and repeat wash twice.

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

Elute proteins

8. Resuspend RFP-Trap®_MA beads in 100 µl 2x SDS-sample buffer.
9. Boil resuspended RFP-Trap®_MA beads for 10 min at 95°C to dissociate immunocomplexes from RFP-Trap®_MA beads. The beads can be magnetically separated 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 magnetic separation. 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.*