

# RFP-Trap®\_A 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

Reagent	Code	Quantity
RFP-Trap®_A	rta-20	20 reactions (0.5 ml slurry)
RFP-Trap®_A	rta-100	100 reactions (2.5 ml slurry)
RFP-Trap®_A	rta-200	200 reactions (5 ml slurry)
RFP-Trap®_A	rta-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 9 µg of recombinant 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

RFP Toolbox	Code
RFP-Trap® protein	rt-250
RFP-Trap®_A Kit	rtak-20
Blocked agarose beads	bab-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

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## Protocol for Immunoprecipitation of RFP-Fusion Proteins using RFP-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 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  $\mu$ 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 supernatant to a pre-cooled tube. Add 300  $\mu$ l 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®\_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 RFP-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 RFP-Trap®\_A beads in 500  $\mu$ l 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 RFP-Trap®\_A beads in 100  $\mu$ l 2x SDS-sample buffer.
9. Boil resuspended RFP-Trap®\_A beads for 10 min at 95°C to dissociate immunocomplexes from RFP-Trap®\_A beads. The beads can be collected by centrifugation at 2.500x g for 2 min 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.*