Rabbit Anti-RFP/mCherry Magnetic Agarose

Properties

Ligand: Anti-RFP Antibody

Binding capacity: No less than 15 µg of recombinant RFP protein per 25 µL bead slurry.

Reactivity: Specifically binds to most common RFP derivatives.

Bead size: 30-100 µm (cross-linked 6 % magnetic agarose beads)

Applications: IP, Co-IP, Protein purification.

Buffer compatibility: See Wash buffer compatibility table.

Stability: Stable storage at 2 to 8 °C for 1 year. Do not freeze!

Required buffer solutions

RIPA buffer: 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.1 % SDS, 1 % NP-40, 0.5 % deoxycholate

Dilution Buffer: 10 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.5 mM EDTA

Wash buffer: 10 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.05 % Tween™ 20, 0.5 mM EDTA.

2x SDS buffer: 100mM Tris-HCl pH 6.8, 20 % glycerol, 4% SDS, 0.04 % bromophenol blue, 10 % β-mercaptoethanol (or 200mM DTT).

Acidic elution buffer: 200 mM glycine pH 2.5.

Neutralization buffer: 1 M Tris pH 10.4.

Wash buffer compatibility table

Buffer ingredients	Max. concentration
DTT	10 mM
Glycerol	30 %
Tween™ 20	tested up to 2 %
NaCl	tested up to 1 M
SDS	0 %

Triton-X100	tested up to 1 %
Urea	4 M

Immunoprecipitation protocol

Take 300μg mammalian cell lysate as an example and start the immunoprecipitation experiment from the protein binding step.

Mammalian cell lysis

1. Selection of lysis buffer
   · For cytoplasmic proteins, resuspend the cell pellet in 300 µL RIPA buffer containing protease inhibitors and 1mM PMSF.
   · For nuclear/chromatin proteins, add DNaseI, MgCl2, protease inhibitor cocktail, and 1mM PMSF to RIPA buffer, then resuspend the cell pellet in 300 µL of RIPA buffer.
2. Place the tube on ice for 30 minutes and mix the suspension every 10 minutes.
3. Centrifuge the cell lysate at 15,000x g for 10 minutes at 4°C. Transfer the supernatant to a pre-chilled EP tube and add 300 µL Dilution buffer. If required, save 50 µL of the diluted lysate for further analysis (input control).

Equilibration & Protein binding

1. Resuspend the beads by gently pipetting up and down or by inverting the tube. Do not vortex the beads.
2. Transfer 25 µL of bead slurry into a 1.5 mL reaction tube. Separate the beads with a Magnetic Separation Rack until the supernatant is clear. Discard the supernatant.
3. Add 100 µL ice-cold Dilution buffer. Separate the beads with a Magnetic Separation Rack until the supernatant is clear. Discard the supernatant. Repeat this step 2-3 times.
4. Add the diluted lysate to the beads and rotate end-over-end for more than 0.5 hour at 25°C.

Note: The specific incubation time can be adjusted according to the binding effect.

Washing

1. Separate the beads with a Magnetic Separation Rack until the supernatant is clear.
2. If required, retain a portion of the supernatant for further analysis and discard the remainder.
3. Add 500 µL wash buffer to resuspend the beads.
4. Separate the beads with a Magnetic Separation Rack until the supernatant is clear. Discard the supernatant.
5. Repeat step 3 & 4 at least twice times.

Elution with 2x SDS buffer

1. Remove the remaining supernatant.
2. Add 20 µL 2x SDS buffer to resuspend the beads.
3. Boil beads for 5 min at +95°C to dissociate immunocomplexes from beads.
4. Separate the beads with a Magnetic Separation Rack.
5. Analyze the supernatant by SDS-PAGE / Western Blot.

Elution with Acidic elution buffer

1. Remove the remaining supernatant.
2. Add 15-25 µL of Acidic elution buffer and pipette up and down continuously for 5-10 minutes at room temperature.
3. Separate the beads with a Magnetic Separation Rack until the supernatant is clear.
4. Transfer the supernatant to a new tube.
5. Immediately neutralize the eluate with neutralization buffer (1/10 of the Acidic elution buffer volume).
6. Repeat this step at least once to increase elution efficiency.

Note: Rabbit Anti-RFP/mCherry Magnetic Agarose beads were not left in the Acidic elution buffer for more than 20 minutes.