

Rabbit Anti-HA tag Agarose beads

Properties

Ligand: Anti-HA Tag Antibody

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

Reactivity: Specifically binds to HA-tag sequence (YPYDVPDYA). Compatible with N-, C-terminal.

Bead size: 45-165 µm (cross-linked 4 % agarose beads)

Applications: Protein purification, IP, Co-IP.

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.

Peptide elution buffer: 150-250 µg/mL HA -peptide.

Wash buffer compatibility table

Buffer ingredients	Max. concentration
NaCl	1 M
DTT	5 mM
B-mercaptoethanol	5 mM
Triton-X100	2%
SDS	0.1%
Urea	4 M

Immunoprecipitation protocol

Take 300 μ g 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 μ L 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 μ L 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 μ L Dilution buffer. If required, save 50 μ L of the diluted lysate for further analysis (input control).

Equilibration & Protein binding

1. Resuspend beads by pipette. **Do not vortex the beads.**
2. Transfer 25 μ L of bead slurry to a 1.5 mL centrifuge tube containing 600 μ L pre-chilled dilution buffer.
3. Centrifuge at 3000xg for 5 minutes at 4°C to collect beads and discard the supernatant.
4. Add the diluted lysate to the centrifuged beads and rotate at 4°C for 2-4 hour.

Note: The amount of lysate needed and incubation time are dependent upon the expression level, type of HA -tagged protein, and type of lysate. Optimization may be required for each specific system.

Washing

1. Centrifuge at 3000x g for 5 minutes at 4°C to pellet the beads.
2. If required, retain a portion of the supernatant for further analysis and discard the remainder.
3. Add 600 μ L wash buffer to resuspend the beads.
4. Centrifuge at 3000x g for 5 minutes at 4°C to pellet the beads and discard the supernatant.
5. Repeat step 3 & 4 at least four times.
6. Last washing step, transfer the beads to a new tube.

Elution with 2x SDS buffer

1. Discard the remaining supernatant.
2. Add 50-100 μ L 2x SDS buffer to resuspend the beads.

3. Heat at 100°C for 5 minutes to separate the immune complexes from the beads.
4. Centrifuge at 3000x g for 2 minutes at 4°C to pellet the beads.
5. Analyze the supernatant by SDS-PAGE/WB.

Note: 1. For Western blot detection. 2.Reducing sample buffer was used and the antibody's 25kDa light chain and 50kDa heavy chain are visible.

Elution with Acidic elution buffer

1. Remove the remaining supernatant.
2. Add 50-100 µL of acidic elution buffer and pipette up and down continuously for 5-10 minutes at room temperature.
3. Centrifuge at 3000 x g for 2 minutes at 4°C to pellet the beads.
4. Transfer the supernatant to a new tube.
5. Immediately neutralize the eluate with 5-10 µL of neutralization buffer.
6. Repeat this step at least once to increase elution efficiency.

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

Elution with Peptide elution buffer

1. Prepare the HA peptide to 150-250µg/mL in PBS.
2. Remove the remaining supernatant from the beads.
3. Add 50-100 µL of the HA peptide and mix at room temperature for 20-30 minutes, or at 4°C for 30-60 minutes.
4. Centrifuge at 3000 x g for 2 minutes at 4°C to pellet the beads.
5. Transfer the supernatant to a new tube.
6. Repeat this step at least once to increase elution efficiency.

Note: Elution at room temperature is more efficient than elution at +4°C. Prewarm buffers for elution at room temperature.