Anti-FLAG (DYKDDDDK) Tag agarose Beads

Properties

Ligand: Anti-FLAG (DYKDDDDK) Tag

Binding capacity: 12-15 µg of recombinant 1xFlag tagged protein per 25µL bead slurry

Reactivity: Specifically binds to flag-tag sequence. Compatible with N-, C-terminal.

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

Applications: IP, Protein purification.

Buffer compatibility: See Wash buffer compatibility table.

Stability: Stable storage at 2 to 8 °C for 1 year.

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.

Peptide elution buffer: 150 µg/mL FLAG-peptide.

Neutralization buffer: 1 M Tris pH 10.4.

Wash buffer compatibility table

Buffer ingredients	Max. concentration
β-mercaptoethanol	10 mM
DTT	10 mM
NaCl	tested up to 1M
SDS	0.2 %
Urea	2 M
Triton-X100	tested up to 0.5 %
Tween™ 20	tested up to 5 %

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 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 beads by pipette. **Do not vortex the beads**.
2. Transfer 25 µL of bead slurry to a 1.5 mL centrifuge tube containing 600 µL 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 1 hour.

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 µL 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 20 µL 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: For Western blot detection.

Elution with Acidic elution buffer

1. Remove the remaining supernatant.
2. Add 40-80µL of acidic elution buffer and incubate for 5-10 min 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 4-8µL of neutralization buffer.
6. Repeat this step at least once to increase elution efficiency.

Elution with Peptide elution buffer

1. Prepare the 1 × FLAG (DYKDDDDK) peptide to 150µg/mL in PBS.
2. Remove the remaining supernatant from the beads.
3. Add 100µL of the 1 × FLAG (DYKDDDDK) 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.