Anti-2-hydroxyisobutyryllysine agarose Beads

Properties

Ligand: Anti-2-hydroxyisobutyryllysine.

Reactivity: With the immobilization of highly specific anti-2-hydroxyisobutyryllysine antibodies, the anti-2-hydroxyisobutyryllysine antibody conjugated agarose beads selectively capture peptides bearing 2-hydroxyisobutyryllysine residues, but does not cross-react with the peptides bearing other structurally similar modified residues.

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

Applications: Peptide immunoaffinity enrichment followed by Mass Spectrometry-based proteomics.

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

Required buffer solutions

IP wash buffer1: 0.5%NP-40,0.1%TritonX-100,1mM EDTA,100 mM NaCl, 50 mM Tris-HCl, pH 8.0.

IP wash buffer2: 100mM NaCl,1 mM EDTA, 50 mM Tris-HCl,pH 8.0.

IP wash buffer3: 50mM ammonium bicarbonate.

Immunoaffinity protocol

1. Resuspend beads by pipette. **Do not vortex the beads**.
2. Transfer 50 µL of bead slurry to a 1.5 mL centrifuge tube containing 0.5 mL pre-chilled PBS.
3. Centrifuge at 1000xg for 1 minutes at 4°C to collect beads and discard the supernatant. Repeat twice.
4. Dissolve 2 mg of desalinated peptides in 1 mL PBS.
5. Remove any possible precipitates in peptide solution by centrifuging at 12,000 x g for 10 min at 4°C.
6. Add the peptide solution to the centrifuged beads and incubate at 4°C overnight with gentle end-over-end rotation.

Washing

1. Centrifuge at 1000x g for 1 minutes at 4°C to pellet the beads.
2. Wash beads with 0.5 mL IP wash buffer 1 by inverting tube 15 times. Centrifuge at 1000x g for 1 minutes at 4°C to pellet the beads and discard the supernatant. Repeat at least two times.
3. Wash beads with 0.5 mL IP wash buffer 2 by inverting tube 15 times. Centrifuge at 1000x g for 1 minutes at 4°C to pellet the beads and discard the supernatant. Repeat at least two times.

4. Wash beads with 0.5 mL IP wash buffer 3 by inverting tube 15 times. Centrifuge at 1000x g for 1 minutes at 4°C to pellet the beads and discard the supernatant. Repeat at least two times.

Elution with 10%HAC

1. Discard the remaining supernatant.
2. Add 100 µL Elution buffer(10%HAC) to resuspend the beads.
3. Incubate for 15 min by end-over-end rotating at room temperature.
4. Centrifuge at 1000 x g for 1 minutes at 4°C to pellet the beads. Spin down beads and transfer eluates into a new tube.
5. Repeat twice and combine all three eluates.