

## Anti-O-GlcNAc agarose Beads

### Properties

**Ligand:** Anti-O-GlcNAc.

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

**Bead size:** 45-165  $\mu\text{m}$  (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  $\mu\text{L}$  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  $\mu$ L 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.