INSTRUCTIONS

Amine Magnetic Beads

Catalog Number: FE02001 FE02002

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【Introduction】

EmerTher® Amine Magnetic Beads are nano-superparamagnetic beads coated with primary amine functional groups, which can be used to covalently conjugate primary amine or carboxy-containing ligands. After coupling, the beads can be separated from the solution using magnet for downstream experiments, such as enzymatic reactions or immunopurification of other large molecules.

【Product Specifications】

Diameter： 500 nm
Magnetic response rate: >30 emu/g
pH stability： 4-12
Storage： 20% ethanol
Binding capacity: 50-500 μg protein per mg amine magnetic beads

【Product Content】

Catalogue Number	Amount of Beads (mg)
FE02001	20
FE02002	100

【Storage】

Shipped at room temperature. Stored at 2-8°C, 2 years.

【Coupling Protocol】

The following protocol provides general guidelines for the coupling of proteins to EmerTher Amine Magnetic Beads. The protocol is scalable. Optimization of the coupling conditions (protein concentration, bead concentration, coupling buffer, pH, and incubation time) for the ligand of interest is recommended.

A. Notes:

1. The following protocol is an example for coupling primary amine-containing ligands to EmerTher Amine Magnetic Beads. EmerTher Amine Magnetic Beads can also couple with carboxy-containing ligands and general protocols for such reaction are applicable.
2. Ionic strengths of the coupling buffers are critical to obtain a higher coupling efficiency rate. The coupling buffers should be at minimal ionic strengths and should not contain any amino (e.g. Tris) or carboxyl groups (e.g. acetate and citrate). But the wash or storage buffers can contain amino or carboxyl groups.
3. The protein concentration should be optimized. Too low a protein concentration may result in bead crosslinking.
4. Solutions containing glutaraldehyde or pyridine are volatile and noxious. Please perform operations with these solutions in a chemical fume hood.
5. Do not freeze, dry, or centrifuge at a high speed during use or storage of beads. Otherwise, this may decrease the binding capacity of the beads.

B. **Additional materials required**:

1. Coupling Buffer: 10 mM pyridine (Add 0.8 ml of pyridine to 900 ml of water. Adjust the pH to 6 with HCl. Add water to a final volume of 1L.)
2. 5% Glutaraldehyde solution: add 5 ml of 25% aqueous glutaraldehyde solution to 20 ml of Coupling Buffer.
3. Quenching Solution: 1.0 M glycine (Dissolve 7.5 g of glycine in 90 ml of water. Adjust the pH to 8 with 10 N NaOH. Add water to a final volume of 100 ml.)

4. Washing Buffer: 0.01 M Tris base containing 0.15 M NaCl, 0.1%(w/v) BSA, 0.001 M EDTA, sodium salt and 0.1%(w/v) sodium azide (Dissolve 1.21 g of Tris base, 8.7 g of NaCl, 1g of BSA, 0.37 g of EDTA, sodium salt and 1.0 g of sodium azide in 900 ml of water. Adjust the pH to 7.4 with HCl. Add water to a final volume of 1 L.)
5. Protein solution: prepared in Coupling Buffer. Protein concentration is typically 1-10 mg/ml.
6. A magnetic stand and a rotator

C. Bead activation

1. Gently mix the magnetic beads thoroughly before use by shaking.
2. Place 1 mg of magnetic beads into a microcentrifuge tube.
3. Place the tube into a magnetic stand, collect the beads and discard the supernatant.
4. Wash the beads three times with Coupling Buffer (1 ml each time) by magnetic separation.
5. Re-suspend the beads by adding 400 µl of 5% Glutaraldehyde and shake. Incubate at room temperature for 3 hr with gentle rotation.
6. Separate beads using a magnet, remove the supernatant.
7. Wash beads three times with 1 ml Coupling Buffer to remove unreacted glutaraldehyde.

D. Coupling of protein

1. Add 200 µl protein solution into the tube containing activated beads and mix well by shaking. Incubate for 24 hr at room temperature or at 4 °C with gentle rotation.
2. Separate beads using a magnet, remove the supernatant.
3. Add 800 µl of Quenching Solution into the tube. Shake to suspend the beads. Gently shake for 30 min at room temperature.
4. Wash the beads with 1 ml Washing Buffer three times.
5. Suspend the beads with a desired volume of Washing Buffer or in a buffer compatible with the attached protein. Store at 4º C until ready for use.