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BcMag Amine-Terminated Magnetic Beads

Introduction

BcMag Amine-Terminated Magnetic Beads are supplied as an aqueous suspension of magnetic iron oxide particles. The beads are sophistically coated to provide primary amino groups. Proteins can be covalently attached to BioMag amino-terminal magnetic beads with retention of biological activities, by the reagents used for preparing affinity supports that the solid phase terminates with primary amino groups.

Size: ~1 um or 5 u m
Concentration: 50mg/ml in 1 mM ETDA, pH 7.0
Store at 4° C. DO NOT FREEZE

Protocol for Coupling of Protein

A. Buffer Preparation

Notes:

1. The ionic strengths of the coupling buffers are critical to obtain the high coupling efficiency rate. The coupling buffers should be at minimal ionic strengths, and should not contain any amino (e.g. Tris) or carboxyl group (e.g. acetate, citrate). But the wash or storage buffers can contain amino or carboxyl groups.
2. Prepare buffer solution in a chemical fume hood because glutaraldehyde or pyridine is volatile and noxious.

- **Coupling Buffer: 10 mM pyridine**
  Add 800ìl pyridine to 900 ml of ddH_2O.
  Adjust to pH 6.0 with HCl. Adjust the final volume to 1L with ddH_2O
- **5% Glutaraldehyde:**
  Add 5.0 ml of 25% glutaraldehyde to 20 ml of Coupling Buffer.
- **Reaction Stop buffer: 1M Glycine**
  Dissolve 7.5 g Glycine in 90 ml of dd H_2O.
  Adjust to pH 8.0 with 10N NaOH. Adjust the final volume to 100 ml with ddH_2O
- **Wash Buffer: 10 mM Tris base, 0.15 M NaCl, 0.1%(w/v) BSA, 1 mM EDTA, 0.1% sodium azide**
  Dissolve 1.21g Tris base, 8.7g NaCl, 1.0 g BSA, 0.37g EDTA, sodium salt, 1.0 g sodium azide in 900ml ddH20. Adjust to pH 7.4 with HCl. Adjust the final volume to 1L with ddH2O.

B. Bead activation

1. Transfer 10 ml of completely suspended magnetic beads to a 50ml conical tube.
2. Add 30m coupling buffer into the tube, resuspend the beads by vigorously shaking, and then insert the tube into a magnetic separator. Let stand until the supernatant is clear. Aspirate the supernatant and discard.

Note:

Magnetic separators are commercially available from Bioclone Inc.: BcMag separator-2 for holding two individual 1.5 ml centrifuge tube, Cat. No. MS-01, BcMag separator-6 for holding six individual 1.5 ml centrifuge tubes, Cat. No. MS-02, BcMag separator-centrifuge tubes, Cat. No. MS-02; BcMag separator-24 for holding twenty-four individual 1.5 ml centrifuge tubes, Cat. No. MS-03, BcMag separator-50 for holding one 50 ml and one 15 ml centrifuge centrifuge tube,Cat. No. MS-04

4. Resuspend the magnetic beads by adding 20 ml of 5% Glutaraldehyde and shake vigorously. Leave at room temperature for 3 hr with gentle rotation.
5. Put the tube into the magnetic separator. Let stand until the supernatant is clear. Carefully aspirate the supernatant and discard.
6. Wash beads three times with coupling buffer as described in step 2 to remove unreacted glutaraldehyde.

C. Coupling of Protein:

1. Prepare protein solution by adding 30-100 mg protein into 10 ml coupling buffer and mix very well.

Note:

For some expensive proteins, such as monoclone antibodies, the supplied concentration cannot reach the required 2.5-10mg/ml. To ensure high efficient coupling, the BSA should be added to the protein solution to bring protein concentration to the required level.

2. Add the protein solution into the tube containing activated beads (step B6) and Mix well by vigorously shaking. Leave reaction for 24 hr at room temperature with gentle rotation.
3. When the reaction is finished, put the tube into the magnetic separator. Let stand until the supernatant becomes clear. Carefully aspirate the supernatant and discard.
4. Add 40ml of reaction stop buffer into the tube. Shake vigorously to suspend the beads. Gently shake for 30 min at room temperature.
5. Put the tube into the magnetic separator. Let stand until the supernatant becomes clear. Carefully remove the supernatant and discard.
6. Add 30ml of wash buffer or desired storage buffer into the tube. Shake to suspend the beads. Then put back into the magnetic separator. Let stand until the supernatant is clear. Carefully aspirate supernatant and discard.

Note:

Some noncovalent adsorption unavoidably occurs during coupling of protein. Noncovalent adsorption can be greatly decreased in wash steps following the coupling. Since noncovalent adsorption is variable for different proteins and different applications, the user should optimize the wash conditions to minimize the noncovalent adsorption for individual application. Usually, more strength wash buffers can be used in wash steps, including high salt (1M NaCl), mildly acidic or basic buffers, wash at higher temperature, more wash steps. However, magnetic beads may be unstable when dissociation of noncovalent attached proteins.

7. Repeat step (step 6) two times.
8. Suspend the beads with desired volume of storage buffer. Store at 4° C