7965 Silverton Ave. Suite 1309
San Diego, CA 92126

BioClone Inc.

Tele: 858-547-8349
Fax: 858-547-8314

BcMag Carboxy-Terminated Magnetic Beads

Introduction

BcMag Carboxy-Terminated Magnetic Beads are supplied as an aqueous suspension of magnetic iron oxide particles. The beads are sophistically coated to provide free carboxyl groups. Proteins and nucleic acids can be covalently attached to BcMag carboxy-terminal magnetic beads with retention of biological activities, by the reagents used for preparing affinity supports that the solid phase terminates with a carboxyl group.

Size: ~1 um or 5 um
Concentration: 20mg/ml in ddH2O
Store at 4° C. DO NOT FREEZE

Protocol for Coupling of Protein

A. Buffer Preparation

Note:
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 groups (e.g. acetate, citrate). But the wash or storage buffers can contain amino or carboxyl groups.

- Coupling Buffer: 10 mM potassium phosphate, 0.15 M NaCl, pH 5.5.
- Coupling agent solution: 0.057% EDCI. Freshly prepared. EDC [1-ethyl-3 (3-dimethyaminopropyl) carbodiimide, Sigma] by dissolving 57mg EDC in 100 ml ddH2O. Use immediately after preparation because this solution is unstable.
- Wash Buffer: 10 mM Tris base, 0.15 M NaCl, 0.1%(w/v) BSA, 1mM EDTA, 0.1% sodium azide, pH 7.5.

B. Procedure

1. Wash BioMag Beads with Coupling Buffer

a. Transfer 10 ml of suspended BioMag Carboxyl-Terminated Magnetic Beads suspension to a 50ml tube.

b. Add coupling buffer into the tube to a final volume of 30ml, shake vigorously to suspend the beads, 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-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 tube, Cat. No. MS-04.

c. Repeat (step 1b) three times.

d. Suspend the particles in 10ml of coupling buffer.

2. Coupling of Protein

a. Prepare 10 ml of protein solution (1mg/ml) with ddH2O and mix well.

b. Add 4ml of coupling agent (EDC) solution into the tube containing washed and resuspended beads (Step 1d). Shake to mix well.

c. Combine the protein solution (step 2a) with the magnetic beads (Step 2b) and mix well by shaking. Leave reaction for 24 hr at room temperature with gentle rotation. Maintain the pH between 4.5-6.0 with 0.1N HCl during coupling.

3. Remove Uncoupled Protein

a. When the reaction is finished, put the tube into the magnetic separator. Let stand until the supernatant becomes clear. Carefully aspirate supernatant and discard.

b. 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 condition 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 noncolvent attached proteins.

c. Repeat (step 3b) two times.

d. Suspend the beads with desired volume of storage buffer. Store at 4° C