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BcMag Hydrazide-Modified Magnetic Beads

Product Description

BcMag Hydrazide-modified Magnetic beads are uniform superparamagnetic 1um (or 5 um) diameter beads with hydrazide surface groups. It is widely used to covalently couple aldehyde- or ketone-containing ligands such as antibody, lectin, glycoprotein, carbohydrates and etc. through the formation of stable hydrazone linkages. Such coupling method is a powerful way to immobilize proteins and leave critical active sites free.

Size: ~1 um (or 5 um)
Concentration: 30mg/ml in PBS, pH 7.0, 0.02 % NaN_3

Store at 4ºC. DO NOT FREEZE

Protocol for Coupling of Protein

A. Buffer Preparation

- **Coupling Buffer:** 0.1 M sodium acetate buffer, pH5.6

  (1) 0.1 M acetic acid: 580 ul made to 100 ml

  (2) 0.1 M sodium acetate, anhydrous: 820 mg/100 ml
  Mix 4.8 ml of 0.1 M acetic acid and 45.2 ml of 0.1 M sodium acetate solution and adjust the final volume to 100 ml with ddH_2O. Adjust the final pH to 5.6 with 0.1 N HCl or NaOH.
- **Storage Buffer:** Phosphate Buffer saline (PBS), pH 7.4, 0.02% NaN_3
  (1) potassium phosphate dibasic: 1.82 g/L
  (2) sodium phosphate monobasic: 0.22 g/L
  (3) sodium chloride: 8.76 g/L
  (4) NaN_3 0.2 g/L
  Bring to final volume of 1 L with ddH_2O. Adjust the final pH to 7.4 with 1 M HCl or NaOH.
- **Blocking buffer:** 67 mM D-glyceraldehyde in coupling buffer
  Dissolve 6 mg D-glyceraldehyde in coupling buffer
- **Oxidation reagent:** sodium meta-periodate ($NaIO_4$)

B. Oxidation of Glycoprotein

Note: The reaction is light sensitive and should be performed in the dark.

1. Dissolve or dilute 5-10 mg glycoprotein in 1 ml coupling buffer.
2. Add the protein solution to an amber vial containing 2 mg sodium meta-periodate (final concentration 10 mM). Swirl gently to dissolve the oxidizing agent.
3. Incubate the sample **in the dark** at room temperature for 30 minutes with gentle rotation.
4. Stop the reaction and remove unreacted $NaIO_4$ by desalting and buffer exchange through Sephadex G-25 column. Equilibrate a 5-ml. Sephadex G-25 column with coupling buffer. Apply the oxidized sample to the column and allow it to enter the gel bed. Apply a 0.5-ml rinse of coupling buffer and allow it also to enter the gel bed. Finally apply 2 ml coupling buffer and collect the eluent.

C. Coupling to BcMag Hydrazide-modified Magnetic Beads

1. Transfer 10 mg of completely suspended magnetic beads to a microcentrifuge tube and insert the tube into a magnetic separator. Wait 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.

2. Wash the beads by adding 1 ml coupling buffer and resuspend the beads by vigorously shaking. Insert the tube into a magnetic separator. Let stand until the supernatant is clear. Aspirate the supernatant and discard.
3. Repeat (step 2) three times.
4. Resuspend the magnetic beads by adding 750 ul coupling buffer.
5. Mix the magnetic beads with 250 ul oxidized protein solution and incubate for a minimum of 6 hours at room temperature.

Note: Coupling efficiencies hydrazide-modified magnetic beads depend on the structure and the size of the target glycoprotein. The user should empirically optimize the concentration of the protein. We recommend starting with 100-250 ug/ml for 10 mg of 1um beads or 25-50 ug/mlfor 10 mg of 5 um beads.

6. Wash beads three times with coupling buffer as described in step 2.
7. Add 200 ul Blocking Buffer to the washed magnetic beads and mix gently for 30-60 minutes.
8. Wash beads 10 times with 1 ml storage buffer as described in step 2.
9. Resuspend the beads in storage buffer to desired concentration.
10. Store at 4ºC until used.