

## BcMag Aldehyde-Modified Magnetic Beads

### Product Description

BcMag Aldehyde-Modified Magnetic Beads are uniform superparamagnetic 1  $\mu$ m (or 5  $\mu$ m) diameter beads with aldehyde surface groups. It is widely used to covalently couple ligands containing amine group.

Size: ~1 $\mu$ m (or 5  $\mu$ m)

Concentration: 30 mg/ml in PBS, pH 7.0, 0.02 %  $\text{NaN}_3$

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 other nucleophiles. But the wash or storage buffers can contain amino.
2. Prepare buffer solution in a chemical fume hood because sodium cyanoborohydride is very toxic.

- **Coupling Buffer:** 0.1 M sodium phosphate, pH 7.0

Dissolve 8.62 g of sodium phosphate dibasic and 5.42 of sodium phosphate monobasic: in 800 ml ddH<sub>2</sub>O. Adjust the final pH to 7.0 with 0.1 N HCl. Adjust the final volume to 1 L with ddH<sub>2</sub>O.

- **Storage Buffer:** Phosphate Buffer saline (PBS), pH 7.4, 0.02%  $\text{NaN}_3$

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|----------------------------------|----------|
| (1) potassium phosphate dibasic: | 1.82 g/L |
| (2) sodium phosphate monobasic:  | 0.22 g/L |
| (3) sodium chloride:             | 8.76 g/L |
| (4) $\text{NaN}_3$               | 0.2 g/L  |

Bring to final volume of 1 L with ddH<sub>2</sub>O. Adjust the final pH to 7.4 with 1 M HCl or NaOH.

- **Coupling reagent:** sodium cyanoborohydride ( $\text{NaCNBH}_3$ )

#### B. Coupling to BcMag Aldehyde-modified Magnetic Beads

1. Transfer 10 mg of completely suspended magnetic beads to a microcentrifuge tube. 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.

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. Dissolve or dilute appropriate amount of protein in 1 ml coupling buffer.

**Note:** Coupling efficiencies to aldehyde-modified magnetic beads depend varies from protein to protein. The user should empirically optimize the concentration of the protein. We recommend starting with 100-250  $\mu$ g/ml for 10 mg of 1 $\mu$ m beads or 25-50  $\mu$ g/ml for 10 mg. of 5  $\mu$ m beads.

5. Add the protein solution to the washed beads. Resuspend the magnetic beads and mix very well.
6. Add 6 mg  $\text{NaCNBH}_3$  to the beads solution and mix the magnetic beads very well. Incubate with continuous rotation at room temperature overnight.
7. Wash beads 10 times with 1 ml ddH<sub>2</sub>O as described at step 2.
8. Resuspend the beads in storage buffer to desired concentration.
9. Store at 4°C until used.