



BcMag™ Sulphydryl-Terminated-Magnetic Beads

Introduction

BcMag™ Sulphydryl-Terminated- Magnetic Beads are 1µm uniform, silica-coated superparamagnetic beads. The Sulphydryl-Terminated- Magnetic Beads are specially designed for conjugation of protein/peptides modified by SMCC (Sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate). BcMag™ Long-Arm Sulphydryl-Terminated-Magnetic Beads are recommended for conjugating small peptides. SMCC contains an amine-reactive N-hydroxysuccinimide (NHS ester) and a sulphydryl-reactive maleimide group. NHS esters react with primary amines at pH 7-9 to form stable amide bonds. Maleimides react with sulphydryl groups at pH 6.5-7.5 to form stable thioether bonds. The procedure is quick and simple for sample preparation without the need for laborious repeat pipetting and centrifuging.

Product Characteristics

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| Composition | Silica-coated iron oxide | |
| Bead Size | 1µm diameter; 5µm diameter | |
| Number of Beads | ~1.7 x 10 ⁸ beads (1µm beads) /mg ; ~5 x 10 ⁷ beads (5µm beads) /mg | |
| Surface Area | ~100 m ² /g | |
| Magnetization | ~40 EMU/g | |
| Type of Magnetization | Superparamagnetic | |
| Effective Density | 2.5 g/ml | |
| Formulation | Lyophilized Powder | |
| Ligand Density | 1µm Sulphydryl-Terminated-Magnetic Beads | ~250 µmole(1µm beads) / g of Beads |
| | 5µm Sulphydryl-Terminated-Magnetic Beads | ~200 µmole (5µm beads) / g of Beads |
| | 1µm Long-Arm Sulphydryl-Terminated-Magnetic Beads | ~220 µmole(1µm beads) / g of Beads |
| | 5µm Long-Arm Sulphydryl-Terminated-Magnetic Beads | ~185 µmole(1µm beads) / g of Beads |
| Storage | Upon receipt store at 4°C | |

Protocol

Materials Required:

- **Conjugation buffer:** PBS, PH 7.2
 1. Dissolve the following in 800ml distilled H₂O.
 - 8g of NaCl
 - 0.2g of KCl
 - 1.44g of Na₂HPO₄
 - 0.24g of KH₂PO₄
 2. Adjust pH to 7.2. Adjust volume to 1L with additional distilled H₂O.
 3. Sterilize by autoclaving
- **SMCC** (Sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (Pierce, cat# 22360 22122) or Sulfo-SMCC (Pierce, cat# 22122)
- **Magnetic Separator (for manual operation):** Based on sample volume, user can choose one of the following magnetic Separator: BcMag separator-2 for holding two individual 1.5 ml centrifuge tubes (Cat. # MS-01); BcMag separator-6 for holding six individual 1.5 ml centrifuge tubes (Cat. # MS-02); BcMag separator-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat. # MS-03); BcMag separator-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Cat.# MS-04)

Protein /Peptide Preparation:

1. Dialyse the protein/Peptides against 50 volumes of PBS, pH 7.2, 5mM EDTA.
2. Add the appropriate amount of SMCC to the protein solution and mix very well.
Notes: Prepare SMCC solution (3.7mg/ml in DMF. If the SMCC does not completely dissolve, place the tube in a 50°C water bath for several minutes.) Add 100 ul of SMCC solution to 1 ml protein solution (10mg/ml). or 50 ul of SMCC solution to 1 ml protein solution (<1mg/ml).



3. Incubate at + 4°C for 2 hours or room temperature for 30 minutes.
4. Remove free SMCC and unmodified proteins on a Sephadex G15 column. Elute with PBS buffer. The elution of the protein-SMCC can be monitored at 206 nm with a spectrophotometer.

Magnetic Beads Preparation:

Note: Weigh, suspend the magnetic beads with PBS (Concentration: 30mg/ml), disperse the beads by vigorously vortexing and store at 4°C. *Shake the bottle to completely resuspend the Magnetic Beads before use.*

1. Shake the bottle to resuspend the BcMagTM Sulfhydryl-Terminated- Magnetic Beads thoroughly.
2. Transfer 100µl-300 µl of the Beads (30 mg/ml) to a tube. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
3. Remove the tube and resuspend the beads thoroughly with 200µl PBS buffer. Leave the tube at room temperature for 2-3 minutes. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
4. Repeat step 3 two times.
5. Incubate the Magnetic Beads with 1 ml of DTT (dithiothreitol 3 mg/ml) for 15 minutes at room temperature, and wash the Beads three times with PBS buffer.
6. Mix the reduced beads with the protein-SMCC conjugate, and incubate for 12 hours at +4°C.
7. Wash the beads and resuspend in the desired buffer.