1 µm Hi-Sur Mag Maleimide Activated Beads Conjugation Protocol

Introduction

Ocean Nanotech's Hi-Sur Mag Maleimide Activated Beads are superparamagnetic beads with larger surface area than Mono Mag (1 µm) to ensure their higher binding capacity than Mono Mag (1 µm). The beads are used to specifically conjugate thiol containing ligands with low non-specific binding.

Briefly, the magnetic beads are activated using Sulfo-SMCC (sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) followed by conjugation to thiol groups that are present on the target protein/ligands. The protocol shown below has been used to successfully conjugate bovine serum albumin, streptavidin and immunoglobulin to Ocean Nanotech's magnetic beads.

Reagents Required

- Magnetic Beads: 1 µm Hi-Sur Mag Maleimide Activated Beads (Product ID: HM1000)
- Coupling Buffer: CB200
- Storage Buffer: SB100

Materials Required

- Target Ligands with Thiol Group
- Magnetic Separator (Product ID: SuperMag Multitube Separator, Supplier: Ocean Nanotech)
- 1.5 mL Microcentrifuge Tubes

Critical Notes Before You Start

- Any other thiol containing molecules in the protein solution, including protein stabilizers, will compete with the conjugation reaction.
- Allow the magnetic beads and the protein to come to room temperature before dissolving them.
- Dissolve the targeted proteins in the coupling buffer. If the targeted protein is already suspended in buffer, such as PBS buffer, this solution could be used directly for conjugation.
- For any vortex steps, vortex at maximum speed to ensure mixing.

Protocol

A. Magnetic Beads Concentration Explanation

1. "2.5 mg magnetic beads" does not mean "2.5 mg lyophilized powder". For example, the weight percentage of the magnetic beads in the lyophilized powder is 5% and 2.5 mg magnetic beads is needed, you will need 50 mg lyophilized powder.

$$\frac{\text{2.5 mg magnetic beads}}{\text{5\% (weight percentage)}} = \text{50 mg lyophilized powder}$$

B. Protein Preparation

1. Use ~0.15 mg protein per 1 mg beads. You may calculate the ligand volume from the concentration.
2. For example, for 2.5 mg beads, you will need 0.375 mg protein. Therefore, if the protein concentration is 1 mg/mL, you will need 0.375 mL protein.

$$\frac{\text{0.375 mg protein}}{\text{1 mg/mL (protein concentration)}} = \text{0.375 mL protein}$$

C. Oligonucleotide or peptides preparation

1. Use ~20 nmol oligonucleotides or peptides per 1 mg beads. You may calculate the ligand volume from the concentration.
2. For example, for 2.5 mg beads, you will need 50 nmol Oligonucleotides or peptides.
3. Oligonucleotide can be coupled to the beads via the 5' or 3' after thiol (SH) modification.

D. Conjugation Procedure

1. Weigh out 2.5 mg magnetic beads (50 mg lyophilized powder) into a 1.5 mL microcentrifuge tube. Add 1 mL coupling buffer to the tube.
2. Vortex the tube and make sure that the magnetic beads are completely resuspended in the solution.
3. Add 0.375 mL thiolated protein (1 mg/mL in coupling buffer) or 50 nmol Oligonucleotides/peptides to the magnetic beads. React at 4 °C or room temperature overnight with continuous mixing.
4. Transfer the magnetic beads suspension in a magnetic separator and allow the magnetic particles to separate (~1 to 2 minutes).
5. Remove the supernatant and add 1 mL storage buffer. Re-suspend the magnetic beads with vortex or sonication.
6. Repeat steps #4 and #5 three times.
7. The third resuspension is the purified protein conjugated magnetic beads. The final product can be stored for more than 12 months in the storage buffer at 2-8°C.