1 µm Hi-Sur Mag Amine Beads Conjugation w/ Sulfo-SMCC Kit Protocol

Introduction

Ocean Nanotech's Hi-Sur Mag Amine 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.

Kit Components and Storage

Each kit contains reagents for 5 reactions (based on 0.4 mL aliquot of magnetic beads)

Kit Components	Quantity	Storage
Magnetic Beads (HA1001)	2 mL (10 mg/mL)	2 to 8 °C, do not freeze
Activation Buffer (AB200)	10 mL	2 to 8°C
Coupling Buffer (CB200)	15 ml	2 to 8°C
Storage Buffer (SB100)	30 mL	2 to 8°C
Sulfo-SMCC	20 mg	-20°C

Materials Required

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

Critical Notes Before You Start

- This protocol is good for 5 reactions per 2 mL magnetic beads (10 mg/mL concentration). Each reaction is based on 0.4 mL aliquot of magnetic beads.
- Resuspend the magnetic beads solution before use.
- Any other thiol containing molecules in the protein solution, including protein stabilizers, will compete with the conjugation reaction.
- Allow the Sulfo-SMCC 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.

Protocol

A. 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 4 mg beads, you will need 0.6 mg protein. Therefore, if the protein concentration is 1 mg/mL, you will need 0.6 mL protein.

$$\frac{0.6 \text{ mg protein}}{1 \text{ mg/mL (protein concentration)}} = 0.6 \text{ mL protein}$$

B. 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 4 mg beads, you will need 80 nmol Oligonucleotides or peptides.
3. Oligonucleotide can be coupled to the beads via the 5' or 3' after thiol (SH) modification.

C. Sulfo-SMCC Solution Preparation

1. Weigh out 2.5 mg Sulfo-SMCC into a microcentrifuge tube. Each tube is good for one reaction use only and should be prepared only before immediate use. After an aliquot of the Sulfo-SMCC solution, do not use the remaining Sulfo-SMCC solution in the tube.
2. Add 0.1 mL DMSO into the preweighed Sulfo-SMCC tube and mix well to dissolve the solids.
3. The desired concentration for Sulfo-SMCC is 25 mg/mL.

D. Conjugation Procedure

1. Aliquot 0.4 mL of the magnetic beads (10 mg/mL) into a 1.5 mL microcentrifuge tube. Place the tube into a magnetic separator.
2. When the supernatant is clear, remove the supernatant. Add 1 mL activation buffer to the magnetic beads and vortex the solution for 15 seconds.
3. Add 0.04 mL Sulfo-SMCC (25 mg/mL in DMSO) to the magnetic beads solution.
4. React at room temperature for 1 hour with continuous mixing.
5. Place tube into the magnetic separator and allow the activated magnetic beads to separate. Remove the supernatant and add 0.5 mL coupling buffer. Re-suspend the magnetic beads with vortex or sonication.
6. Place the tube into the magnetic separator and allow the activated magnetic beads to separate.
7. Wash the activated beads one more time with 0.5 mL coupling buffer (repeat step #5 and #6).
   Note: This purification step should be done as soon as possible.
8. Place the tube into the magnetic separator and allow the magnetic beads to separate.
9. Remove the supernatant and add 0.4 mL coupling buffer to the magnetic beads. Re-suspend the magnetic beads by vortex or sonication.
   Note: The magnetic beads should be completely resuspended before adding protein.
10. Add 0.6 mL targeted protein (1 mg/mL in coupling buffer) or 80 nmol oligonucleotides/peptides to the magnetic beads. React at 4 °C or room temperature overnight with continuous mixing.
11. Place the microcentrifuge tube in a magnetic separator and allow 1 to 2 minutes for the magnetic particles to separate.
12. Remove the supernatant and add 1 mL storage buffer. Re-suspend the magnetic beads with vortex or sonication.
13. Repeat steps #11 and #12 three times.
14. The third resuspension is the purified protein conjugated magnetic beads. The final product can be stored up to 12 months in the storage buffer at 2-8°C.