1 µm Hi-Sur Mag Carboxylic Acid Beads Conjugation Protocol

Introduction

Ocean Nanotech's Hi-Sur Mag Carboxylic Acid 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 primary amine-containing ligands with low non-specific binding.

Briefly, the magnetic beads are activated using EDC/Sulfo-NHS followed by conjugation to amine 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.

One Step Protocol

Reagents Required

- Magnetic Beads: 1 µm Hi-Sur Mag Carboxylic Acid Beads (Product ID: HC1000)
- EDC (1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride)
- Activation Buffer: AB100
- Quenching Buffer: QB100
- Storage Buffer: SB100

Materials Required

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

Critical Notes Before You Start

- Resuspend the magnetic beads solution before use.
- Any other amine containing molecules (e.g. BSA) in the protein solution, including protein stabilizers, will compete with the conjugation reaction.
- Allow the EDC and the protein to come to room temperature before dissolving them.
- Dissolve the targeted proteins in the activation buffer. If the targeted protein is already suspended in buffer, such as PBS buffer, this solution could be used directly for conjugation. Meanwhile, please choose a large reaction tube with a suitable size (5 mL) to carry out the conjugation if the reaction volume is over 1.5 ml.
- For any vortex steps, vortex at maximum speed to ensure mixing.

A. Protein Preparation

1. Use ~0.1 mg pure protein per 1 mg beads. You may calculate the protein volume from the concentration.
2. For example, for 4 mg beads, you will need 0.4 mg protein. Therefore, if the protein concentration is 1 mg/mL, you will need 0.4 mL protein.

$$\frac{0.4 \text{ mg protein}}{1 \text{ mg/mL (protein concentration)}} = 0.4 \text{ mL protein}$$

B. Ligands preparation

1. Use ~20 nmol ligands (e.g. oligonucleotides) 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 ligands (e.g. oligonucleotides) or peptides.
3. Oligonucleotide can be coupled to the beads via the 5' or 3' after amino (NH_2) modification.

C. EDC Solution Preparation

1. Weigh out 5 mg EDC into one tube.
2. The EDC solution should be prepared only before immediate use. After an aliquot of the EDC solution, do not use the remaining EDC solution in the tube.
3. Add 0.5 mL DI water into the preweighed EDC tube and mix well to dissolve the solids. The desired concentration for EDC is 10 mg/mL.

D. Conjugation Procedure

1. Aliquot 0.08 mL of the magnetic beads (50 mg/mL) into a 1.5 mL microcentrifuge tube and place the microcentrifuge tube in a magnetic separator.
2. Remove the supernatant with a pipette until the supernatant is clear. Add 0.4 mL activation buffer to the resuspend the magnetic beads.
3. Add 0.04 mL EDC solution (10 mg/ml) to the magnetic beads solution. React at room temperature for 15 minutes with continuous mixing.
   Note: The amount of EDC and targeted ligands may need to be optimized to obtain desired binding capacity.
4. Add 0.4 ml protein (1 mg/ml in activation buffer) or 80 nmol oligonucleotides (in activation buffer) to the magnetic beads and vortex the mixture for 30 seconds.
5. React at room temperature for 2.5 hours with continuous mixing.
6. Add 0.1 mL quenching buffer to the magnetic beads suspension and react at room temperature for 30 minutes with continuous mixing.
7. Place the tube in a magnetic separator and wait 1 to 2 minutes for the beads to separate.
8. Remove the supernatant and add 1 mL storage buffer. Re-suspend the magnetic beads with vortex or sonication.
9. Repeat steps #7 and #8 three times. Resuspend the magnetic beads in storage buffer.
10. The third resuspension is the purified magnetic beads. The final product can be stored for more than 12 months in the storage buffer at 2-8°C.

Two Steps Protocol

Reagents Required

- Magnetic Beads: 1 μm Hi-Sur Mag Carboxylic Acid Beads (Product ID: HC1001, HC1002, HC1003)
- EDC (1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride)
- Sulfo-NHS (N-hydroxysulfosuccinimide)
- Activation Buffer: AB100
- Quenching Buffer: QB100
- Storage Buffer: SB100

Materials Required

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

Critical Notes Before You Start

- Resuspend the magnetic beads solution before use.
- Any other amine containing molecules (e.g. BSA) in the protein solution, including protein stabilizers, will compete with the conjugation reaction.
- Allow the EDC/Sulfo-NHS and the protein to come to room temperature before dissolving them.
- Dissolve the targeted proteins in the activation buffer. If the targeted protein is already suspended in buffer, such as PBS buffer, this solution could be used directly for conjugation. Meanwhile, please choose a large reaction tube with a suitable size (5 mL) to carry out the conjugation if the reaction volume is over 1.5 ml.

- For any vortex steps, vortex at maximum speed to ensure mixing.

A. Protein Preparation

1. Use ~0.1 mg pure protein per 1 mg beads. You may calculate the protein volume from the concentration.
2. For example, for 4 mg beads, you will need 0.4 mg protein. Therefore, if the protein concentration is 1 mg/mL, you will need 0.4 mL protein.

$$\frac{0.4 \text{ mg protein}}{1 \text{ mg/mL (protein concentration)}} = 0.4 \text{ mL protein}$$

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 amino (NH_2) modification.

C. EDC/Sulfo-NHS Solution Preparation

1. Weigh out 5 mg EDC into one tube, and weigh out 5 mg Sulfo-NHS into another tube.
2. The EDC/NHS solution should be prepared only before immediate use. After an aliquot of the EDC solution and Sulfo-NHS solution, do not use the remaining EDC solution and Sulfo-NHS solution in the tube.
3. Add 0.5 mL DI water into the preweighed EDC tube and mix well to dissolve the solids. The desired concentration for EDC is 10 mg/mL.
4. Add 0.5 mL DI water into the preweighed Sulfo-NHS tube and mix well to dissolve the solids. The desired concentration for Sulfo-NHS is 10 mg/mL.

D. Conjugation Procedure

1. Aliquot 0.08 mL of the magnetic beads (10 mg/mL) into a 1.5 mL microcentrifuge tube and place the microcentrifuge tube in a magnetic separator.
2. Remove the supernatant with a pipette until the supernatant is clear. Add 1 mL activation buffer to resuspend the magnetic beads.
3. Add 0.1 mL Sulfo-NHS solution (10 mg/mL) and 0.1 mL EDC solution (10 mg/mL) to the magnetic beads solution.
   Note: The amount of EDC and targeted ligands may need to be optimized to obtain desired binding capacity.
4. React at room temperature for 15 minutes with continuous mixing.
5. Place tube into the magnetic separator and allow the activated magnetic beads to separate. Remove the supernatant and add 0.4 mL activation buffer to re-suspend the magnetic beads.
   Note: The magnetic beads should be completely resuspended before adding targeted ligands. Vortex or sonicate the magnetic beads if necessary.
6. Add 0.4 mL targeted protein (1 mg/mL in activation buffer) or 80 nmol oligonucleotides/peptides (in activation buffer) to the magnetic beads. React at room temperature for 2.5 hours with continuous mixing.
7. Add 0.1 mL quenching buffer to the magnetic beads suspension and React at room temperature for 30 minutes with continuous mixing.
8. Place the tube into the magnetic separator and wait until the supernatant is clear.
9. Remove the supernatant and add 1 mL storage buffer. Re-suspend the magnetic beads with vortex or sonication.
10. Repeat steps #8 and #9 three times. Resuspend the magnetic beads in storage buffer.
11. 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.

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