50 nm Super Mag Carboxylic Acid Beads Conjugation Protocol

Introduction

Ocean Nanotech's carboxyl functionalized magnetic beads are uniform superparamagnetic beads with high density of carboxyl group on the surface. 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 Conjugation Protocol

Reagents Required

- Magnetic Beads: 50 nm Super Mag Carboxylic Acid Beads (Product ID: SC0050)
- 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

Critical Notes Before You Start

- This protocol is good for 4 reactions per 1 mL magnetic beads (10 mg/mL concentration). Each reaction is based on 0.25 mL aliquot of magnetic beads.
- 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.
- For any vortex or sonication steps, vortex at maximum speed to ensure mixing. Bath sonication is highly recommended.

A. Protein Preparation

1. Use ~0.1 mg protein per 1 mg beads. You may calculate the ligand volume from the concentration.
2. For example, for 2 mg beads, you will need 0.2 mg protein. Therefore, if the protein concentration is 1 mg/mL, you will need 0.2 mL protein.

$$\frac{0.2 \text{ mg protein}}{1 \text{ mg/mL (protein concentration)}} = 0.2 \text{ mL protein}$$

B. 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. Each tube is good for one reaction use only and 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 activation buffer 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 activation buffer into the preweighed Sulfo-NHS tube and mix well to dissolve the solids. The desired concentration for Sulfo-NHS is 10 mg/mL.

C. Conjugation Procedure

1. Aliquot 0.25 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 0.4 mL activation buffer to the resuspend the magnetic beads.
3. Firstly add 12.5 μL Sulfo-NHS solution (10mg/mL) and then add 12.5 μL EDC solution (10mg/mL) to the magnetic beads solution.
4. React at room temperature for 15 mins with continuous mixing. Add 0.25 ml targeted protein (1 mg/ml in activation buffer or PBS buffer) to the magnetic beads.
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 2 to 8 hours (depending on the strength of the magnetic field of the magnetic separator) 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 #6 and #7 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 Conjugation Protocol

Reagents Required

- Magnetic Beads: 50 nm Super Mag Carboxylic Acid Beads (Product ID: SC0050)
- 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
- Desalting Column: illustra NAP-5 Columns (Product ID: 17-0835-01, Supplier: GE Healthcare)

Critical Notes Before You Start

- This protocol is good for 4 reactions per 1 mL magnetic beads (10 mg/mL concentration). Each reaction is based on 0.25 mL aliquot of magnetic beads.
- 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.
- For any vortex or sonication steps, vortex at maximum speed to ensure mixing. Bath sonication is highly recommended.

D. Protein Preparation

1. Use ~0.1 mg protein per 1 mg beads. You may calculate the ligand volume from the concentration.
2. For example, for 2 mg beads, you will need 0.2 mg protein. Therefore, if the protein concentration is 1 mg/mL, you will need 0.2 mL protein.

$$\frac{0.2 \text{ mg protein}}{1 \text{ mg/mL (protein concentration)}} = 0.2 \text{ mL protein}$$

E. 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. Each tube is good for one reaction use only and 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 activation buffer 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 activation buffer into the preweighed Sulfo-NHS tube and mix well to dissolve the solids. The desired concentration for Sulfo-NHS is 10 mg/mL.

F. Conjugation Procedure

1. Aliquot 0.25 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 0.4 mL activation buffer to the resuspend the magnetic beads.
3. Firstly add 12.5 µL Sulfo-NHS solution (10mg/mL) and then add 4.5 µL EDC solution (10mg/mL) to the magnetic beads solution.
4. React at room temperature for 15 mins with continuous mixing.
5. Equilibrate a NAP-5 column with 1 mL activation buffer for 3 times. Transfer the 0.5 mL activated beads to the equilibrated NAP-5 column to remove the excess EDC/Sulfo-NHS. Collect 1 mL eluted magnetic beads into a 1.5 mL microcentrifuge tube.
6. Add 0.25 ml targeted protein (1 mg/ml in activation buffer or PBS buffer) to the magnetic beads.
7. React at room temperature for 2.5 hours with continuous mixing.
8. Add 0.1 mL quenching buffer to the magnetic beads suspension and React at room temperature for 30 minutes with continuous mixing.
9. Place the tube in a magnetic separator and wait 2 to 8 hours (depending on the strength of the magnetic field of the magnetic separator) for the beads to separate.
10. Remove the supernatant and add 1 mL storage buffer. Re-suspend the magnetic beads with vortex or sonication.
11. Repeat steps #6 and #7 three times. Resuspend the magnetic beads in storage buffer.
12. 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.