1 µm Mono 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.

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 (MC1001)	2 mL (10 mg/mL)	2 to 8 °C, do not freeze
Activation Buffer (AB100)	15 mL	2 to 8°C
Quenching Buffer (QB100)	1 mL	2 to 8°C
Storage Buffer (SB100)	30 mL	2 to 8°C
EDC	20 mg	-20°C
Sulfo-NHS	20 mg	-20°C

One Step Protocol

Reagents Required

- Magnetic Beads: 1 µm Mono Mag Carboxylic Acid Beads (Product ID: MC1001)
- EDC (1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride)
- Activation Buffer
- Quenching Buffer
- Storage Buffer

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 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 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 steps, vortex at maximum speed to ensure mixing.

A. Protein Preparation

1. Use ~0.05 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 0.2 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. Ligands preparation

1. Use ~10 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 40 nmol ligands (e.g oligonucleotide).
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 0.4 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 resuspend the magnetic beads.
3. Add 0.02 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 may need to be optimized to obtain desired binding capacity.
4. Add 0.2 ml protein (1 mg/ml in activation buffer) or 40 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 Mono Mag Carboxylic Acid Beads (Product ID: MC1001)
- EDC (1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride)
- Sulfo-NHS (N-hydroxysulfosuccinimide)
- Activation Buffer
- Quenching Buffer
- Storage Buffer

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

A. Protein Preparation

1. Use ~0.05 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 0.2 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. Oligonucleotide or peptides preparation

1. Use ~10 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 40 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 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 0.4 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 resuspend the magnetic beads.
3. Add 0.1 mL Sulfo-NHS solution and 0.1 mL EDC solution to the magnetic beads solution.
   Note: The amount of EDC/Sulfo-NHS 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. Re-suspend the magnetic beads with vortex or sonication.
   Note: The magnetic beads should be completely resuspended before adding protein. Vortex or sonicate the magnetic beads if necessary.
6. Add 0.2 mL targeted protein (1 mg/mL in activation buffer) or 40 nmol oligonucleotides/peptides 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 1 to 2 minutes for the beads to separate.
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 magnetic beads. The final product can be stored for more than 12 months in the storage buffer at 2-8°C.

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