4.5 µm Mono Mag NHS-Activated Beads Conjugation Protocol

Introduction

Ocean Nanotech's NHS activated Mono Mag beads are uniform superparamagnetic beads with narrow size distribution (CV<5%) and high density of NHS groups on the surface. The beads are used to specifically conjugate primary amine-containing ligands with low non-specific binding. 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: 4.5 µm Mono NHS Beads (Product ID: MN4500)
- Resuspension 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 is a recommended protocol that works for 250 mg lyophilized powder contains 12.5 mg magnetic beads. Target protein amount may be needed to adjust to obtain desire binding capacity.
- Any other amine containing molecules (e.g. BSA) in the protein solution, including protein stabilizers, will compete with the conjugation reaction.
- Allow the magnetic beads and all reagents to come to room temperature before dissolving them.
- Dissolve the targeted proteins in the resuspension 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. "100 mg magnetic beads" does not mean "100 mg lyophilized powder". For example, the weight percentage of the magnetic beads in the lyophilized powder is 5% and 10 mg magnetic beads is needed, you will need 200 mg lyophilized powder.

$$\frac{\text{10 mg magnetic beads}}{\text{5\% (weight percentage)}} = \text{200 mg lyophilized powder}$$

B. Protein Preparation

1. Use ~0.03 mg protein per 1 mg beads. You may calculate the ligand volume from the concentration.
2. For example, for 10 mg beads, you will need 0.3 mg protein. Therefore, if the protein concentration is 1 mg/mL, you will need 0.3 mL protein.

$$\frac{\text{0.3 mg protein}}{\text{1 mg/mL (protein concentration)}} = \text{0.3 mL protein}$$

C. Oligonucleotide or peptides preparation

1. Use ~2 nmol oligonucleotides or peptides per 1 mg beads. You may calculate the ligand volume from the concentration.
2. For example, for 10 mg beads, you will need 20 nmol Oligonucleotides or peptides.
3. Oligonucleotide can be coupled to the beads via the 5' or 3' after amino (NH_2) modification.

D. Conjugation Procedure

1. Add 1 mL resuspension buffer to the pre-packed 12.5 mg magnetic beads tube. Re-suspend the magnetic beads with vortex for 15 minutes.
   Note: Do not proceed to the next step without vortexing the beads for 15 minutes.
2. Place tube into the magnetic separator and allow the activated magnetic beads to separate. Remove the supernatant and add 0.5 mL resuspension buffer. Re-suspend the magnetic beads with vortex or sonication.
   Note: The magnetic beads should be completely suspended before adding proteins.
3. Add 0.375 mL targeted protein (1 mg/mL in resuspension buffer) or 25 nmol Oligonucleotides/peptides to the magnetic beads. React at room temperature for 2.5 hours with continuous mixing.
4. Add 0.1 mL quenching buffer to the magnetic beads suspension. React at room temperature for 30 minutes with continuous mixing.
5. Place the tube into a magnetic separator. Remove the supernatant after the supernatant is clear.
6. Add 1 mL storage buffer and re-suspend the magnetic beads with vortex or sonication.
7. Repeat steps #5 and #6 three times. Resuspend the magnetic beads in storage buffer.
8. 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.