50 nm Super Mag NHS-Activated Beads Conjugation Kit Protocol

Introduction

Ocean Nanotech's NHS activated magnetic beads are uniform superparamagnetic beads with 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.

Kit Components and Storage

Each kit contains reagents for 4 reactions (based on 2.5 mg beads/reaction)

Kit Components	Quantity	Storage
Magnetic Beads (SN0051)	10 mg	-20°C
Resuspension 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

Materials Required

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

Critical Notes Before You Start

- All our NHS activated magnetic beads are pre-packaged with 2.5 mg magnetic beads or 50 mg lyophilized powder in each tube. To make sure the highest binding capacity of the lyophilized powder, please use all the lyophilized powder once the tube is open. The NHS functional group will be de-activated when exposed to the air.
- 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. "2.5 mg magnetic beads" does not mean "2.5 mg lyophilized powder". For example, the weight percentage of the magnetic beads in the lyophilized powder is 5% and 2.5 mg magnetic beads is needed, you will need 50 mg lyophilized powder.

$$\frac{\text{2.5 mg magnetic beads}}{\text{5\% (weight percentage)}} = \text{50 mg lyophilized powder}$$

B. Protein Preparation

1. Use ~0.1 mg protein per 1 mg beads. You may calculate the protein volume from the concentration.
2. For example, for 2.5 mg beads, you will need 0.25 mg protein. Therefore, if the protein concentration is 1 mg/mL, you will need 0.25 mL protein.

$$\frac{\text{0.25 mg protein}}{\text{1 mg/mL (protein concentration)}} = \text{0.25 mL protein}$$

C. Oligonucleotide or peptides preparation

1. Use ~50 nmol oligonucleotides or peptides per 1 mg beads. You may calculate the ligand volume from the concentration.
2. For example, for 2.5 mg beads, you will need 125 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 0.25 mL resuspension buffer to the pre-packed 2.5 mg magnetic beads tube. Re-suspend the magnetic beads with continuous mixing for 15 minutes.
2. Add 0.25 mL targeted protein (1 mg/mL in Resuspension Buffer) or 125 nmol oligonucleotides/peptides to the magnetic beads. React at room temperature for 2.5 hours with continuous mixing.
3. Add 0.1 mL quenching buffer to the magnetic beads suspension and React at room temperature for 30 minutes with continuous mixing.
4. Add 1 mL storage buffer to the magnetic beads suspension. Transfer the magnetic beads suspension into a magnetic separator and allow 2 to 8 hours (depending on the strength of the magnetic field of the magnetic separator) for the magnetic particles to separate.
5. Remove the supernatant and add 1 mL storage buffer. Re-suspend the magnetic beads with vortex or sonication.
6. Repeat steps #5 and #6 three times. Resuspend the magnetic beads in storage buffer.
7. 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.