

4.5 μ m Mono Mag Maleimide Activated Beads Conjugation Kit Protocol

Introduction

Ocean Nanotech's maleimide activated magnetic beads are uniform superparamagnetic beads with high density of maleimide on the surface. The beads are used to specifically conjugate thiol containing ligands with low non-specific binding.

Briefly, the magnetic beads are activated using Sulfo-SMCC (sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) followed by conjugation to thiol 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 4 reactions (based on 12.5 mg beads/reaction)

Kit Components	Quantity	Storage
Magnetic Beads (MM4501)	50 mg	-20°C
Coupling Buffer (CB200)	15 mL	2 to 8°C
Storage Buffer (SB100)	30 mL	2 to 8°C

Critical Notes Before You Start

- Any other thiol containing molecules in the protein solution, including protein stabilizers, will compete with the conjugation reaction.
- Allow the magnetic beads and the protein to come to room temperature before dissolving them.
- Dissolve the targeted proteins in the coupling 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. "10 mg magnetic beads" does not mean "10 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.

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

B. 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 10 mg beads, you will need 0.5 mg protein. Therefore, if the protein concentration is 1 mg/mL, you will need 0.5 mL protein.

$$\frac{0.5 \text{ mg protein}}{1 \text{ mg/mL (protein concentration)}} = 0.5 \text{ mL protein}$$

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 thiol (SH) modification.

D. Conjugation Procedure

1. Weigh out 12.5 mg magnetic beads (250 mg lyophilized powder) into a 5 mL microcentrifuge tube. Add 1 mL coupling buffer to the tube.
2. Vortex the tube and make sure that the magnetic beads are completely resuspended in the solution.
3. Add 0.625 mL thiolated protein (1 mg/mL in coupling buffer) or 25 nmol Oligonucleotides/peptides to the magnetic beads. React at 4°C or room temperature overnight with continuous mixing.
4. Transfer the magnetic beads suspension in a magnetic separator and allow the supernatant is clear.
5. Remove the supernatant and add 1 mL storage buffer. Re-suspend the magnetic beads with vortex or sonication.
6. Repeat steps #4 and #5 three times.
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.

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