Absolut Mag™ Amine Magnetic Particles Conjugation Kit, 150 nm, BS3 as Crosslinker Conjugation Protocol

Cat# WHM-K013

Introduction

Absolut Mag™ amine functionalized magnetic particles are uniform superparamagnetic beads with high density of Amine 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 BS3 (bis(sulfosuccinimidyl)suberate) 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 CD's magnetic beads.

Kit Components and Storage

Each kit contains reagents for 5 reactions (based on 0.2 mL aliquot of magnetic beads)

Kit Components	Quantity	Storage
Magnetic Beads (WHM-X015)	1 mL (10 mg/mL)	2 to 8 °C, do not freeze
Coupling Buffer	30 mL	2 to 8°C
Quenching Buffer	2 mL	2 to 8°C
Storage Buffer	50 mL	2 to 8°C
BS3	25 mg	-20°C

Buffer Components

- Coupling Buffer: 10 mM PBS, 0.01% tween 20, pH 7.4
- Quenching Buffer:100 mM Tris-HCl, pH 7.4
- Storage Buffer: 10 mM PBS, 0.05% NaN_3, 0.01% tween 20, pH 7.4

Materials Required

- Target Ligands with Amine Group
- Magnetic Separator
- 1.5 mL Microcentrifuge Tubes

Critical Notes Before You Start

- This protocol is good for 5 reactions per 1 mL magnetic beads (10 mg/mL concentration). Each reaction is based on 0.2 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 BS3 and the protein to come to room temperature before dissolving them.

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- 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. 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.

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

B. Oligonucleotide or peptides preparation

1. Use ~15 nmol oligonucleotides or peptides per 1 mg beads. You may calculate the ligand volume from the concentration.
2. For example, for 2 mg beads, you will need 30 nmol Oligonucleotides or peptides.
3. Oligonucleotide can be coupled to the beads via the 5' or 3' after amino (NH_2) modification.

C. BS3 Solution Preparation

1. Weigh out 5 mg BS3 into a microcentrifuge tube. Each tube is good for one reaction use only and should be prepared only before immediate use. After an aliquot of the BS3 solution, do not use the remaining BS3 solution in the tube.
2. Add 0.5 mL coupling buffer into the preweighed BS3 tube and mix well to dissolve the solids.
3. The desired concentration for BS3 is 10 mg/mL.

D. Conjugation Procedure

1. Aliquot 0.2 mL of the magnetic beads (10 mg/mL) into a 1.5 mL centrifuge tube.
2. Add 0.1 mL coupling buffer to the magnetic beads and vortex the solution for 15 seconds.
3. Add 0.1 mL BS3 (10 mg/mL in coupling buffer) to the magnetic beads solution.
4. React at room temperature for 30 minutes with continuous mixing.
5. Place tube into the magnetic separator and allow the activated magnetic beads to separate. Remove the supernatant and add 0.5 mL coupling buffer. Re-suspend the magnetic beads with vortex or sonication.
6. Place the tube into the magnetic separator and allow the activated magnetic beads to separate.
7. Wash the activated beads one more time with 0.5 mL coupling buffer (repeat step #5 and #6).

   Note: This purification step should be done as soon as possible.
8. Remove the supernatant and add 0.4 mL coupling buffer. Re-suspend the magnetic beads with vortex or sonication.

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Note: The magnetic beads should be completely resuspended before adding protein.

9. Add 0.2 mL targeted protein (1 mg/mL in coupling buffer) or 30 nmol oligonucleotides/peptides. React at room temperature for 2.5 hours with continuous mixing.
10. Add 0.1 mL quenching buffer to the magnetic beads suspension and react at room temperature for 30 minutes with continuous mixing.
11. Place the microcentrifuge tube into a magnetic separator and allow 1 to 2 minutes for the magnetic beads to separate.
12. Remove the supernatant and add 1 mL storage buffer. Re-suspend the magnetic beads with vortex or sonication.
13. Repeat steps #11 and #12 three times.
14. 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.