

Absolut Mag™ Maleimide Activated Magnetic Particles Conjugation Kit, 100 nm

Conjugation Protocol

Cat# WHM-K031

Introduction

Absolut Mag™ Maleimide Activated Magnetic Particles 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 successfully conjugate bovine serum albumin, streptavidin and immunoglobulin to Creative Diagnostics'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 (WHM-K033B)	10 mg	-20°C
Coupling Buffer	30 mL	2 to 8°C
Storage Buffer	50 mL	2 to 8°C

Buffer Components

- Coupling Buffer: 10 mM PBS, 5 mM EDTA, 0.01% Tween 20, pH 7.2
- Storage Buffer: 10 mM PBS, 0.05% NaN₃, 0.01% Tween 20, pH 7.4

Materials Required

- Target Ligands with Thiol Group
- Magnetic Separator
- 5 mL Reaction Tubes

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. "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.

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

B. Protein Preparation

1. Use ~0.20 mg proteins per 1 mg beads. You may calculate the ligand volume from the concentration.
2. For example, for 2.5 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 ~40 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 100 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 2.5 mg magnetic beads (50 mg lyophilized powder) into a 5 mL reaction 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 0.5 mL thiolated protein (1 mg/mL in coupling buffer) or 100 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 magnetic particles to separate (~2 to 5 minutes).
5. Remove the supernatant and add 2 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.

WARRANTIES AND DISCLAIMER

The Alpha Biobeads product ("Product") is warranted to operate or perform in conformance with published Product specifications at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products **are supplied for research use only**. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non-infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.