CD Creative Diagnostics®

Absolute Mag™ NHS-Activated Magnetic Particles Conjugation Kit, 1 μm

Conjugation Protocol

Cat# WHM-K044

Introduction

Absolute Mag™ NHS-Activated Magnetic Particles 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 Absolute Mag™ NHS-Activated Magnetic Particles.

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-X025)	10 mg	-20°C
Resuspension Buffer	30 mL	2 to 8°C
Quenching Buffer	2 mL	2 to 8°C
Storage Buffer	50 mL	2 to 8°C

Buffer Components

- Resuspension Buffer: 25 mM MES, 0.01% Tween 20, pH 6.0
- Quenching Buffer: 100 mM, Tris-HCl, pH 7.4
- Storage Buffer: 10 mM PBS, 0.01% tween 20, 0.05% NaN_3, pH 7.4

Materials Required

- Target Ligands with Amine Group
- Magnetic Separator
- 1.5 mL Microcentrifuge 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 ligand 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 ~10 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 25 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 2.5 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 0.25 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 (1 to 2 minutes).
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.