CD Creative Diagnostics®

Absolut Mag™ Carboxyl Magnetic Particles Conjugation Kit, 200 nm Conjugation Protocol
Cat# WHM-K028

Introduction

Absolute Mag™ Carboxyl Magnetic Particles, 200 nm are uniform superparamagnetic beads with high density of carboxyl 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 EDC/Sulfo-NHS 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 Absolute Mag™ Carboxyl Magnetic Particles, 200 nm.

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-X009)	1 mL (10 mg/mL)	2 to 8 °C, do not freeze
Activation Buffer	30 mL	2 to 8°C
Quenching Buffer	2 mL	2 to 8°C
Storage Buffer	50 mL	2 to 8°C
EDC	25 mg	-20°C
Sulfo-NHS	25 mg	-20°C

One Step Conjugation Protocol

Reagents Required

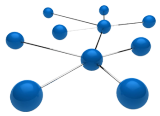
- Magnetic Beads: Absolute Mag™ Carboxyl Magnetic Particles, 200 nm
- EDC (1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride)
- Activation Buffer: 25 mM MES, 0.01% Tween 20, pH 6.0
- Quenching Buffer: 100 mM, Tris-HCl, pH 7.4
- Storage Buffer: 10 mM PBS, 0.01% tween 20, 0.05% NaN_3, pH 7.4

Materials Required

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

Critical Notes Before You Start

- 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 EDC and the protein to come to room temperature before dissolving them.
- Dissolve the targeted proteins in the activation 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.

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.

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

B. Oligonucleotide preparation

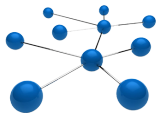
1. Use ~25 nmol oligonucleotides or peptides per 1 mg beads. You may calculate the ligand volume from the concentration.
2. For example, for 2 mg beads, you will need 50 nmol Oligonucleotides or peptides.
3. Oligonucleotide can be coupled to the beads via the 5' or 3' after amino (NH_2) modification.

C. EDC Solution Preparation

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

D. Conjugation Procedure

1. Aliquot 0.2 mL of the magnetic beads (10 mg/mL) into a 1.5 mL microcentrifuge tube and add 0.2 mL activation buffer to the microcentrifuge tube.
2. Add 0.01 mL EDC solution to the magnetic beads solution.
3. React at room temperature for 15 mins with continuous mixing. Add 0.2 ml targeted protein (1 mg/ml in activation buffer) or 50 nmol oligonucleotides in activation buffer to the magnetic beads.
4. React at room temperature for 2.5 hours with continuous mixing.
   Note: The amount of EDC and targeted ligands may be need to be optimized to obtain desired binding capacity.
5. Add 0.1 mL quenching buffer to the magnetic beads suspension and React at room temperature for 30 minutes with continuous mixing.
6. Place the tube in a magnetic separator and wait 2 to 5 min for the beads to separate.
7. Remove the supernatant and add 1 mL storage buffer. Re-suspend the magnetic beads with vortex or sonication.
8. Repeat steps #6 and #7 three times. Resuspend the magnetic beads in storage buffer.
9. The third resuspension is the purified magnetic beads. The final product can be stored for more than 12 months in the storage buffer at 2-8°C.



Two Steps Conjugation Protocol

Reagents Required

- Magnetic Beads: Absolute Mag™ Carboxyl Magnetic Particles, 200 nm
- EDC (1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride)
- Sulfo-NHS (N-hydroxysulfosuccinimide)
- Activation 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

- 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 EDC/Sulfo-NHS and the protein to come to room temperature before dissolving them.
- Dissolve the targeted proteins in the activation 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.

A. Protein Preparation

1. Use ~0.1 mg proteins 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.

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

B. Oligonucleotide or peptides preparation

1. Use ~25 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 50 nmol Oligonucleotides or peptides.
3. Oligonucleotide can be coupled to the beads via the 5’ or 3’ after amino (NH_2) modification.

C. EDC/Sulfo-NHS Solution Preparation

1. Weigh out 5 mg EDC into one tube, and weigh out 5 mg Sulfo-NHS into another tube.
2. Each tube is good for one reaction use only and should be prepared only before immediate use. After an aliquot of the EDC solution and Sulfo-NHS solution, do not use the remaining EDC solution and Sulfo-NHS solution in the tube.
3. Add 0.5 mL DI water into the preweighed EDC tube and mix well to dissolve the solids. The desired concentration for EDC is 10 mg/mL.
4. Add 0.5 mL DI water into the preweighed Sulfo-NHS tube and mix well to dissolve the solids. The desired concentration for Sulfo-NHS is 10 mg/mL.

D. Conjugation Procedure

1. Aliquot 0.2 ml of the magnetic beads (10 mg/ml) into a 1.5 ml microcentrifuge tube and add 0.2 mL activation buffer to the microcentrifuge tube.
2. Add 0.04 mL Sulfo-NHS solution and 0.04 ml EDC solution to the magnetic beads suspension.
3. React at room temperature for 15 minutes with continuous mixing.
4. Place tube into the magnetic separator and allow the activated magnetic beads to separate. Remove the supernatant and add 0.5 mL activation buffer. Re-suspend the magnetic beads with vortex or sonication.
   Note: The magnetic beads should be completely resuspended before adding protein.
5. Add 0.2 mL targeted protein (1 mg/mL in activation buffer) or 50 nmol oligonucleotides/peptides to the magnetic beads. React at room temperature for 2.5 hours with continuous mixing.
   Note: If the protein has been dissolved in PBS buffer, the protein could be used directly without buffer exchange.
6. Add 0.1 mL quenching buffer to the magnetic beads suspension and React at room temperature for 30 minutes with continuous mixing.
7. Place the microcentrifuge tube in a magnetic separator and wait 2 to 5 minutes for the beads to separate from the supernatant.
8. Remove the supernatant and add 1 mL storage buffer. Re-suspend the magnetic beads with vortex or sonication.
9. Repeat steps #7 and #8 three times. Resuspend the magnetic beads in storage buffer.
10. 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.