Absolute Mag™ Carboxylic Acid Iron Oxide Nanoparticles Conjugation Protocol

Introduction

Absolute Mag™ Carboxylic Acid Iron Oxide Nanoparticles are superparamagnetic particles with high density of carboxyl group on the surface. The nanoparticles are used to specifically conjugate primary amine-containing ligands with low non-specific binding.

Briefly, the magnetic nanoparticles 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™ Carboxylic Acid Iron Oxide Nanoparticles.

One Step Conjugation Protocol

Reagents Required

- Iron Oxide Nanoparticles (Product ID: WNM-X013, WNM-X014, WNM-X015, WNM-X016, WNM-X017, WNM-X018)
- EDC (1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride)
- Activation Buffer: 25 mM MES, pH 6.0
- Coupling buffer: 10 mM PBS buffer pH 7.4
- Quenching Buffer: 100 mM, Tris-HCl, pH 7.4
- Storage Buffer: 10 mM PBS, pH 7.4

Materials Required

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

Critical Notes Before You Start

- This protocol is good for 5 reactions per 2 mL magnetic nanoparticles (5 mg/mL concentration). Each reaction is based on 0.2 mL aliquot of magnetic nanoparticles.
- Resuspend the magnetic nanoparticles 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.
- If there is sodium azide such as 0.1% in solution, it is better to remove it
- Conjugation efficiency is related to protein concentration
- EDC concentration should be around 0.1-0.25mg/ml in final reaction solution for effective conjugation.

A. Protein Preparation

- The reaction molar ratio of the protein to nanoparticles listed in table. You can choose suitable reaction ratio for your experiment.
- Disperse protein in the coupling buffer in 2 mg/mL

B. Oligonucleotide preparation

1. Use ~5 nmol oligonucleotides or peptides per 1 mg nanoparticles. You may calculate the ligand volume from the concentration.
2. For example, for 2 mg nanoparticles, you will need 10 nmol Oligonucleotides or peptides.
3. Oligonucleotide can be coupled to the nanoparticles via the 5' or 3' after amino (NH_2) modification.

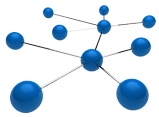
C. EDC Solution Preparation

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 nanoparticles (5 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 nanoparticles solution.
3. React at room temperature for 15 mins with continuous mixing. Add targeted protein to the magnetic nanoparticles.
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 nanoparticles suspension and React at room temperature for 30 minutes with continuous mixing.
6. Remove unconjugated protein or oligonucleotide with the methods suggested in the table below.

Particle size (nm)	Molar number per 1 mg IO Nanomole	Suggested molar reaction ratio(protein/IO)	Antibody amount/mg IO (MW:150,000) (mg)	Purification Methods
5	6.9	2	2.06	ultrahigh speed centrifugation (90,000 rpm)
10	0.86	10	1.29	ultrahigh speed centrifugation (75,000 rpm) or Magwire
15	0.27	15	0.61	ultrahigh speed centrifugation (60,000 rpm),Magwire or Magnetic separator
20	0.11	25	0.40	ultrahigh speed centrifugation (60,000 rpm), Magwire or Magnetic separator



25	0.058	35	0.31	ultrahigh speed centrifugation (60,000 rpm), Magwire or Magnetic separator
30	0.034	50	0.26	ultrahigh speed centrifugation (60,000 rpm), Magwire or Magnetic separator

7. Resuspend the magnetic nanoparticles in storage buffer with desired concentration.

Two Steps Conjugation Protocol

Reagents Required

- Magnetic Nanoparticles: WNM-X014, WNM-X015, WNM-X016, WNM-X017, WNM-X018
- EDC (1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride)
- Sulfo-NHS (N-hydroxysulfosuccinimide)
- Activation Buffer: 25 mM MES, 0.01% Tween 20, pH 6.0
- Coupling buffer: 10 mM PBS buffer pH 7.4
- Quenching Buffer: 100 mM, Tris-HCl, pH 7.4
- Storage Buffer: 10 mM PBS, pH 7.4

Materials Required

- Target ligands with Amine Group
- Magnetic Separator
- 1.5 mL Microcentrifuge Tubes
- NAP-10 column (GE 17-0854-02)
- Magwire column

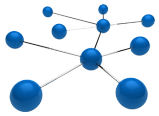
Critical Notes Before You Start

- This protocol is good for 5 reactions per 2 mL magnetic nanoparticles (5 mg/mL concentration). Each reaction is based on 0.2 mL aliquot of magnetic nanoparticles.
- Resuspend the magnetic nanoparticles 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. The reaction molar ratio of the protein to nanoparticles listed in table. You can choose suitable reaction ratio for your experiment.
2. Disperse protein in the coupling buffer in 2 mg/mL

b) Oligonucleotide or peptides preparation



3. Use ~5 nmol oligonucleotides or peptides per 1 mg nanoparticles. You may calculate the ligand volume from the concentration.
4. For example, for 2 mg nanoparticles, you will need 10 nmol Oligonucleotides or peptides.
5. Oligonucleotide can be coupled to the nanoparticles via the 5′ or 3′ after amino (NH2) modification.

F 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 nanoparticles (5 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 Sulfo-NHS solution and 0.01 ml EDC solution to the magnetic nanoparticles suspension.
3. React at room temperature for 15 minutes with continuous mixing.
4. Separate unreacted EDC/NHS by NAP-10 column
5. Add targeted protein to the magnetic nanoparticles eluted from the column.
6. React at room temperature for 2.5 hours with continuous mixing.
   Note: The amount of EDC and targeted ligands may need to be optimized to obtain desired binding capacity.
7. Add 0.1 mL quenching buffer to the magnetic nanoparticles suspension and React at room temperature for 30 minutes with continuous mixing.
8. Remove unconjugated protein or oligonucleotide with the methods suggested in the table above.
9. Resuspend the magnetic nanoparticles in storage buffer with desired concentration.