# Absolute Mag™ Azide Iron Oxide Nanoparticles Click Chemistry Kit Conjugation Protocol

**Cat# WNM-X098K--WNM-X101K**

## Introduction

Absolute Mag™ Azide Iron Oxide Nanoparticles are uniform nanoparticles with a high density of azide groups on the surface. The nanoparticles are used to specifically conjugate alknye containing ligands with low non-specific binding via click chemistry strategy.

The target protein/biomolecules are first activated with DBCO-NHS followed by reacting with azide functionalized magnetic nanoparticles via click chemistry. The protocol shown below has been used successfully to conjugate streptavidin and fluorescent dyes with Absolute Mag™ Azide Iron Oxide Nanoparticles.

## Materials Required for Conjugation Procedures

- Azide Iron Oxide Nanoparticles (WNM-X098--WNM-X101, 1 mg/mL)
- Coupling Buffer (10 mM PBS, pH 7.4)
- PD10 desalting column (Product#17-0851-01, GE Healthcare Life Sciences)
- DBCO-Sulfo-NHS
- Target Protein with Amine Groups
- Microcentrifuge Tubes
- Vortex Mixer (Product ID: 58816-121, Supplier: VWR)
- Insert Retainer (Product ID: 58816-132, Supplier: VWR)
- Micro-Tube Holder (Product ID: 12620-876, Supplier: VWR)

## Critical Notes before You Start

- A PD10 desalting column should be equilibrated with coupling buffer for 3 times (3 mL coupling buffer each time).
- Any other amine containing molecules (e.g. BSA) in the protein solution, including protein stabilizers, will compete with the conjugation reaction.
- Allow the DBCO-sulfo-NHS and the protein to come to room temperature before dissolving them.
- This protocol uses a targeted protein with molecular weight around 50,000 Dalton as the example.
- Dissolve the targeted protein in PBS buffer at pH 7.4.
- For any vortex steps, vortex at maximum speed to ensure mixing.
- For any mixing steps, place the microcentrifuge tube in the foam micro-tube holder and power on the vortex mixer.

**Protocol with DBCO-NHS**

**A. DBCO-NHS Solution Preparation**

1. Weight out 1 mg of DBCO-Sulfo-NHS in a microcentrifuge tube.
2. Add 0.1 mL coupling buffer into the microcentrifuge tube and mix well to dissolve the solids.
3. The desired concentration for DBCO-Sulfo-NHS is 10 mg/mL.
4. The DBCO-Sulfo-NHS is not stable in the aqueous solution. Each DBCO-Sulfo-NHS solution should be prepared only before immediate use and is good for one reaction only. After an aliquot of the DBCO-Sulfo-NHS solution, do not use the remaining solution in the tube.

**B. Conjugation Procedure with DBCO-Sulfo-NHS as Cross-Linker**

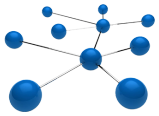
1. Add certain amount of DBCO-Sulfo-NHS (10 mg/mL in coupling buffer) to into a 2 mL microcentrifuge tube with 0.3 mL coupling buffer.

| Particle size (nm) | Amount of DBCO-Sulfo-NHS (μL) |
|---|---|
| 5 | 10 |
| 10 | 7 |
| 20 | 6 |
| 30 | 5 |

2. Add certain amount of target protein (1 mg/mL in coupling buffer) to DBCO-Sulfo-NHS suspension.

| Particle size (nm) | Amount of target biomolecules (μL) |
|---|---|
| 5 | 500 |
| 10 | 430 |
| 20 | 360 |
| 30 | 300 |

3. React at room temperature for 1 hour with continuous mixing.
4. Aliquot 1 mL of the magnetic nanoparticles with azide groups (1 mg/mL) into a 2 mL microcentrifuge dark tube with 0.5 mL of coupling buffer.
5. Add the DBCO-NHS-target biomolecule conjugates into the azide iron oxide nanoparticles
suspensions.
6. React at room temperature for 3 hours with continuous mixing on the rotating wheel.



7. After three hours, add certain amount of coupling buffer to make the final volume at 2.5 mL.
8. Transfer 2.5 mL activated beads to the equilibrated PD-10 column to remove the excess molecules. Collect 3.5 mL eluted magnetic beads into a 5 mL reaction tube.
9. Add certain amount of coupling buffer depending on the desired concentration.

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