



Azide Functionalized Magnetic Nanoparticles Conjugation Kit

Introduction:

Ocean NanoTech's azide functionalized magnetic nanoparticles are uniform nanoparticles with a high density of azide groups on the surface. The nanoparticles are used to specifically conjugate alkyne containing ligands with low non-specific binding via click chemistry strategy.

The target protein/biomolecules are firstly activated with DBCO-NHS followed by reacting with azide functionalized magnetic nanoparticles via click chemistry. This protocol has been used successfully to conjugate streptavidin and fluorescent dyes with Ocean NanoTech's azide-magnetic nanoparticles.

Conjugation Kits (Catalog # SZK) components:

- Magnetic Nanoparticles (IAZ, 1 mg/mL), 1 ml
- DBCO-Sulfo-NHS, 5 mg
- Coupling, 10 ml
- Storage Buffer, 10 mL

Materials required but not provided:

- Target Protein with Amine Groups
- SuperMag Multitube Separator (Catalogue # SuperMag Multitube Separator™)
- 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:

- 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 target protein with molecular weight around 50,000 Dalton as an example.
- Dissolve the target protein in Coupling Buffer.
- 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.

Calculation of each component:

IO Size	IO amount	Protein amount MW: 50,000	DBCO amount	Ratio of DBCO/Protein	Ratio of Protein/IO
10 nm	1 mg	0.86 mg	0.14 mg	20	20
20 nm	1 mg	0.36 mg	0.06 mg	20	65
30 nm	1 mg	0.3 mg	0.05 mg	20	176

A: DBCO-NHS Solution Preparation

1. Weight out 1 mg of DBCO-Sulfo-NHS in a microcentrifuge tube.
2. Add 0.1 mL water 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 before immediate use and is 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 target protein (1 mg/mL in coupling buffer) to a 2 mL microcentrifuge tube DBCO-Sulfo-NHS suspension.

Particle size (nm)	Amount of target biomolecules (µL)
10	860
20	360
30	300

2. Add certain amount of DBCO-Sulfo-NHS (10 mg/mL) into above protein solution

Particle size (nm)	Amount of DBCO-Sulfo-NHS (µL)
10	14
20	6
30	5

3. React at room temperature for 1 hour with continuous mixing.
4. Add 1 mL of the IAZ nanoparticles with azide groups (1 mg/mL) into above protein-DBCO solution.
5. React at room temperature for 3 hours with continuous mixing on the rotating wheel.
6. Protein conjugated nanoparticles could be purified by Multi-tube Separator to remove unreacted proteins
7. Suspension the purified nanoparticles in Storage buffer.

C: Storage:

- All the solutions in the kit should be stored at 4°C. The DBCO-Sulfo-NHS should be stored at -20°C.
- The conjugates can be stored in Storage Buffer at 4°C.



Ocean NanoTech, LLC
7964 Arjons Dr. Ste G, San Diego, CA 92126
Phone : (858) 689-8808; Fax: (858) 689-8809
E-mail : info@oceannanotech.com

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