

## **Amine Iron Oxide Nanoparticles Conjugation Kits (Catalog # IAK)**

Ocean NanoTech's amine functionalized magnetic iron oxide nanoparticles (SHAs) of various diameters (5 nm-30 nm) are available in an easy-to-use kit format to enable researchers to conjugate proteins/ligands of their own choice to these magnetic iron oxide nanoparticles. The kit contains sufficient reagents & components for performing 5 conjugation reactions using 1.25 mg magnetic iron oxide nanoparticles per reaction. Briefly, the magnetic nanoparticles are activated using Sulfo-SMCC (succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate) followed by conjugation to thiol groups that are present on the target protein/ligands.

**IMPORTANT: PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING**

### **Amine Magnetic Iron Oxide Nanoparticles Conjugation Kits (Catalog # IAK) Contents:**

- 1 mL of iron oxide nanoparticles (5 mg/mL, Catalog# SHA) of customer choice
- Sulfo-SMCC, 20 mg (**store at -20 °C upon arrival**).
- CB300, Coupling Buffer, 15 mL.
- QB300, Quenching Buffer, 0.5 mL.
- SB300, Storage Buffer, 15 mL.

### **Materials required but not provided:**

- DMSO (Dimethyl sulfoxide) or DMF (Dimethylformamide)
- Target protein/ligand with thiol groups
- Pipettes for delivering 10 µL to 1 mL volumes
- Vortex mixer capable of securing 1.5 mL tubes for incubation
- Standard laboratory disposables
- -20 °C freezer and 4 °C refrigerator
- SuperMag Multitube Separator (Catalogue # SuperMag Multitube Separator™)
- NAP-10 desalting column
- 1.7 mL low protein binding centrifuge tubes

### **Reagents Preparation:**

*NOTE: Allow all reagents to come to room temperature before starting.*

- Sulfo-SMCC Solution:
  1. Weight out 1 mg Sulfo-SMCC in a microcentrifuge tube.
  2. Add 0.1 mL DMSO (or DMF) into the microcentrifuge tube and mix well to dissolve the solids.
  3. The desired concentration for Sulfo-SMCC is 10 mg/mL.

*Note: The Sulfo-SMCC is not stable in the aqueous solution. Each Sulfo-SMCC solution should be prepared only before immediate use and is good for one reaction only. After an aliquot of the Sulfo-SMCC solution, do not use the remaining Sulfo-SMCC solution.*

### **Conjugation Protocol:**

1. Aliquot 0.25 mL of the magnetic nanoparticles (5 mg/mL) into a 1.7 mL microcentrifuge tube with 0.25 mL coupling buffer.
2. Add certain amount of Sulfo-SMCC (10 mg/mL in DMSO or DMF) to the magnetic nanoparticle suspension depending on the particle size.

Particle size (nm)	Amount of SMCC (mL)
5	0.0625
10	0.0625
15	0.038
20	0.038
25	0.03
30	0.03

3. React at room temperature for 1 hour with continuous mixing.
4. Load all activated iron oxide to the NAP-10 column equilibrated with Coupling Buffer. Add 1.5 mL Coupling Buffer to the column after all nanoparticle solution enter the column. Collect eluted solution (1.5 mL) into a tube labelled as activated iron oxide.
5. Add a certain amount of targeted protein with thiol groups (1 mg/mL) to the magnetic nanoparticles. React at room temperature for 4 hours with continuous mixing.

Particle size (nm)	Amount of Protein (mL)
5	0.375
10	0.375
15	0.25
20	0.25
25	0.19
30	0.19

6. Add 0.05 mL quenching buffer to the magnetic nanoparticle suspension. Mix well and incubate for 30 mins at room temperature with continuous mixing.
7. Magnetic separation method is highly recommended to purify the protein conjugated magnetic nanoparticles with 0.5 or 1 mL storage buffer.

Particle Size	Purification Method
5 nm	Ultracentrifuge, 80,000 rpm, 45-75 minutes
10 nm	SuperMag Multitube Separator, 24-30 hours
15 nm	SuperMag Multitube Separator, 12-18 hours
20 nm	SuperMag Multitube Separator, 8-12 hours
25 nm	SuperMag Multitube Separator, 8-12 hours
30 nm	SuperMag Multitube Separator, 8-12 hours

**Note:** Particles at sizes from 10 nm to 30 nm can also be purified using ultracentrifuge. The rpm and time needed for purification may vary for conjugates with different proteins and will need to be tested and optimized.

8. Resuspend the magnetic nanoparticles in certain amount of storage buffer depending on the desired concentration.

### **Storage:**

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

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