



Carboxyl Iron Oxide Nanoparticles Conjugation Kits (ICK)

Ocean NanoTech's carboxyl terminated magnetic iron oxide nanoparticles (SHPs) 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 and components for performing 5 conjugation reactions using 1 mg magnetic iron oxide nanoparticles per reaction.

Briefly, the magnetic iron oxide nanoparticles are conjugated to amine groups that are present on the target protein/ligand using carbodiimide. The protocol shown below has been used to successfully conjugate bovine serum albumin, streptavidin, and immunoglobulin to Ocean Nanotech's magnetic iron oxide nanoparticles.

IMPORTANT: PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING.

Carboxyl Magnetic Iron Oxide Nanoparticles Conjugation Kits (Catalog # ICK) Contents:

- 1 mL of 5 mg/mL magnetic iron oxide nanoparticles (Catalog# SHP)
- EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide), 20 mg (**Store at -20 °C upon arrival**)
- AB300, Activation Buffer, 10 mL
- QB100, Quenching Buffer, 1 mL
- SB300, Washing/Storage Buffer, 15 mL

Materials required but not provided:

- Target protein/ligand with primary amine 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 # MMS-1.5-8, Ocean NanoTech) or ultracentrifuge
- 1.7 mL low protein binding centrifuge tubes

Reagents Preparation:

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

▪ Protein/Ligand Solution

Dissolve/dilute protein/ligand in activation buffer to 1 mg/ml. If your protein/ligand is in amine containing buffer (such as Tris buffer) or at lower concentration, please use spin column to do buffer exchange (with activation buffer) and concentrating.

Note: Any other amine containing molecules in the protein solution (including protein stabilizers) will compete with the conjugation reaction.

▪ EDC

Weigh out 2.5 mg EDC and add 250 µL Activation Buffer into the tube. Mix well to dissolve the solids, yielding a final concentration of 10 mg/mL EDC.

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

Conjugation Protocol:

1. Aliquot 0.2 mL of the magnetic iron oxide nanoparticles into a low protein binding centrifuge tube and add 0.1 mL Activation Buffer to the magnetic iron oxide nanoparticles.
2. Add 0.5 mL of protein/ligand solution (1 mg/ml). Mix well.
3. Add 200 μ L of the EDC solution (10 mg/mL) into the magnetic iron oxide nanoparticles and protein/ligand solution.

Note: The amount of EDC here is only for reference. The end user should always optimize the EDC input to achieve the optimal performance.

4. React at room temperature for 2 hours with continuous mixing.
5. Add 10 μ L of the Quenching Buffer, mix well and incubate for 30 minutes at room temperature.
6. Remove unconjugated protein with the methods suggested in the table below:

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.

7. Resuspend the magnetic nanoparticles in 0.5 mL washing/storage buffer. Repeat steps 6-7 one more time.
8. Resuspend the magnetic nanoparticles in washing/storage buffer to desired concentration.

Storage:

- All the solutions in the kit should be stored at 4°C. The EDC vial should be stored at -20°C.
- The conjugates can be stored in the Washing/Storage Buffer at 4°C.

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