Absolute Mag™ Carboxylic Acid Iron Oxide Nanoparticles Conjugation Kit Conjugation Protocol

(Catalog # WNM-X014K--WNM-X018K)

Absolute Mag™ Carboxylic Acid Iron Oxide Nanoparticles of various diameters (10 nm-50 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 at least 5 conjugation reactions using 1 mg magnetic iron oxide nanoparticles per reaction. All buffers and nanoparticles in the kits have been autoclaved.

Briefly, the magnetic iron oxide nanoparticles are activated using carbodiimide and N-hydroxysuccinimide followed by conjugation to amine groups that are present on the target protein/ligand. The protocol shown below has been used to successfully conjugate bovine serum albumin, streptavidin and immunoglobulin to Absolute Mag™ Carboxylic Acid Iron Oxide Nanoparticles.

IMPORTANT: PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING.

Carboxylic Acid Iron Oxide Nanoparticles Conjugation Kits contents:

- 5 mg/mL Carboxylic Acid Iron Oxide Nanoparticles (Cat# WNM-X014--WNM-X018), 1 mL
- Pre-weighed mixture of 2 mg EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) and 1 mg NHS (sulfo-N-hydroxysuccinimide) per tube, 5 tubes
- Activation Buffer, 20 mL
- Coupling Buffer, 20 mL
- Quenching Buffer, 0.1 mL
- 10x Wash/Storage Buffer, 5 mL (***NOTE:*** *dilute 10 times with DI water before use*)
- Low Protein Binding Centrifuge Tubes, 5× 1.7 mL

Materials required but not provided:

- Pipettes for delivering 10 uL to 1 mL volumes
- Vortex mixer capable of securing 1.5 mL tubes for incubations

- Standard laboratory disposables
- 4°C Refrigerator
- SuperMag Multitube Separator (Cat# WHK-MS007) and 4.5 mL capacity plastic cuvettes

Reagents Preparation:

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

Protein/Ligand Solution:

Dissolve or dilute protein/ligand with the Coupling Buffer provided to a concentration ≥ 2 mg/mL. Make sure that the protein stock utilized does not contain any molecules with amine or carboxyl groups.

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

EDC & NHS Mixture:

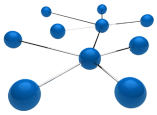
Add 1mL Activation Buffer into the pre-weighed EDAC /NHS mixture tube and mix well to dissolve the solids, yielding a final concentration of 2 mg/ml EDAC and 1 mg/ml NHS.

The EDC / NHS stock solution must be used immediately after preparation!

Conjugation Protocol:

NOTE: It is best to use plastic microcentrifuge tubes of at least 1.5 mL capacity to perform the conjugation reaction.

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 100 uL of the EDC /NHS solution into the magnetic iron oxide nanoparticles solution and mix well. *This gives a final concentration of 0.5 mg/ml EDC, 0.25 mg/ml NHS, which we have determined to be optimal for most conjugation reactions. In the event of precipitation of nanoparticles, the amount of EDC/NHS added may be adjusted to achieve an [EDC] of 0.2 to 0.5 mg/ml at this stage in the reaction.*
3. React at room temperature for 5-10 minutes with continuous mixing.
4. Add 0.4 mL of the Coupling Buffer to the activated magnetic iron oxide nanoparticles, mix well, and immediately add at least 1 mg of protein/ligand contained in a maximum volume of 0.5 mL to the activated magnetic iron oxide nanoparticles mixture and mix well again.



5. React at room temperature for 2 hours with continuous mixing.

NOTE: *conjugation progress can be monitored with agarose gel electrophoresis by running the product alongside unlabeled carboxylic acid iron oxide Nanoparticles after 30 minutes of reaction if it is available at your facility.*

6. Add 10 uL of the Quenching Buffer, mix well and incubate for 10 minutes at room temperature.

7. Transfer the reaction mixture into a plastic cuvette, add 3 mL Wash/Storage Buffer into the cuvette and mix gently with a pipette.

8. Insert the cuvette into the SuperMag Multitube Separator and allow conjugated iron oxide nanoparticles to separate at 4°C.

NOTE: *If using iron oxide nanoparticles of 10 nm–25 nm diameter, then perform this magnetic separation for 10 to 24 hours. For 30 nm-50 nm diameter iron oxide nanoparticles, let separate for 3 to 5 hours. Always visually check the cuvette to make sure that all the iron oxide nanoparticles have separated onto the inner walls of the cuvette.*

9. Carefully aspirate all the liquid from the tube using a suitable pasteur pipette, being careful not to touch the inner walls of the cuvette.

10. Remove the cuvette from the magnetic separator and add 3 mL Wash/Storage Buffer and gently resuspend the magnetic iron oxide nanoparticles using a suitable pipette.

11. Repeat steps # 8 thru #10 one more time and finally resuspend the conjugated magnetic iron oxide nanoparticles with 1 mL of the Wash/Storage Buffer for ~3 months storage at 4°C.

Storage:

- All the solutions in the kit should be stored at 4°C. The pre-weighed EDC/NHS vials should be stored at -20°C.
- The conjugates can be stored for up to 3 months in the Wash/Storage Buffer at 4°C.