



Carboxyl Magnetic Iron Oxide Nanocrystals Conjugation Kits (Catalog # IPK)

Ocean NanoTech's carboxyl terminated magnetic iron oxide nanocrystals of various diameters (10 nm-50 nm) are now available in an easy-to-use kit format to enable researchers to conjugate proteins/ligands of their own choice to these magnetic iron oxide nanocrystals. The kit contains sufficient reagents & components for performing at least 5 conjugation reactions using 1 mg magnetic iron oxide nanocrystals per reaction. Briefly, the magnetic iron oxide nanocrystals are activated using carbodiimide and N-hydroxysuccinimide followed by conjugation to amino 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 these magnetic iron oxide nanocrystals.

IMPORTANT: PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING.

Carboxyl Magnetic Iron Oxide Nanocrystals Conjugation Kits (Catalog # IPK-30-005) contents:

- 1 mL of 5 mg/mL magnetic iron oxide nanocrystals (Catalog# SPP-30-10)
- 5 tubes containing a pre-weighed mixture of 2 mg EDAC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) and 1 mg NHS (sulfo-N-hydroxysuccinimide) per tube.
- Activation Buffer, 20 mL
- Coupling Buffer, 20 mL
- Quenching buffer, 0.1 mL
- Wash/Storage buffer, 10 mL
- 5× 1.5 mL low protein binding centrifuge tubes

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
- Analytical balance capable of accurately weighing at least 2 mg of solids
- Test tubes and standard laboratory disposables
- SuperMAG Magnetic Separation Device (Catalogue # SuperMag SeparatorTM)
- 4.5 mL capacity plastic cuvettes for magnetic washes if using the SuperMag SeparatorTM separation device

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/ligand stock utilized does not contain any contaminating amino or carboxyl buffer ions. The best way to remove such contaminating ions is by the use of desalting columns.

EDAC & 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 EDAC / NHS stock solution must be used immediately after preparation!

10X Wash/Storage Buffer:

Dilute 1mL of the 10X wash/storage buffer concentrate with 9 mL deionized water to give 10 mL of a 1X working solution. This will be sufficient for 1 x 1 mg magnetic iron oxide nanocrystals conjugation.

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 nanocrystals into a 1.5 mL tube and add 0.2 mL activation buffer to the magnetic iron oxide nanocrystals.
2. Add 100 μ L of the EDAC /NHS solution into the magnetic iron oxide nanocrystals solution and mix well. *This gives a final concentration of 0.5 mg/ml EDAC, 0.25 mg/ml NHS, which we have determined to be optimal for most conjugation reactions. In the event of low conjugation efficiency, the amount of EDAC/NHS added may be adjusted to achieve an [EDAC] of 0.2 to 1 mg/ml at this stage in the reaction.*
3. React at room temperature for 5-10 minutes with continuous mixing.
4. Add 0.5 mL of the coupling buffer to the activated magnetic iron oxide nanocrystals, 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 nanocrystals mixture and mix well again.
5. React at room temperature for 2 hours with continuous mixing.*
6. Add 10 μ L of the quenching solution, mix well and incubate for 10 minutes at room temperature.
7. Transfer the reaction mixture into a plastic cuvette, add 3 mL wash/storage into the cuvette and mix gently with a pipette.
8. Insert the cuvette into the SuperMag SeparatorTM magnetic separator and allow conjugated magnetic iron oxide nanocrystals to separate at 4°C.

NOTE: If using magnetic iron oxide nanocrystals of 10 nm–25 nm diameter, then perform this magnetic separation for 10 to 24 hours. For 30 nm-50 nm diameter magnetic iron oxide nanocrystals, let separate for 3 to 5 hours.

Always visually check the cuvette to make sure that all the magnetic iron oxide nanocrystals 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 nanocrystals using a suitable pipette.
 11. Repeat steps # 8 thru #10 one more time and finally resuspend the conjugated magnetic iron oxide nanocrystals with 1 mL of the Wash/Storage buffer for ~3 months storage.
- * Success of conjugation may be verified as early as 30 minutes into the conjugation reaction by performing 1% agarose gel electrophoresis by running the product alongside unlabeled SPP.

Storage:

- All the solutions in the kit should be stored at 4°C. The pre-weighed EDAC /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.

Related Ocean NanoTech Products

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Current versions of product instructions are available at www.oceannanotech.com. For a faxed copy, call 479-431-4130 or 479-751-5500.

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