



Conjugation Protocol

Red Carboxyl Fluorescent Nanoparticles (F61503)

Ocean Nanotech's carboxylated fluorescent nanoparticles are uniform polystyrene beads with intense fluorescent signals. They are used to specifically conjugate primary amine-containing ligands. Briefly, the fluorescent nanoparticles are activated using EDC followed by conjugation to amine groups that are present on the target protein/ligands.

IMPORTANT: PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING.

MATERIALS:

1. Red carboxyl fluorescent nanoparticles, 100 nm (Product ID: F61503)
2. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)
3. N-hydroxysulfosuccinimide (Sulfo-NHS)
4. MES (100 mM, pH 6.0)
5. Coupling Buffer (50 mM Borate, pH 8.5)
6. Quenching Buffer (BSA, 10 mg/mL in 1x PBS)
7. Storage Buffer (50 mM Borate, 0.1% BSA, 0.05% ProClin 300, pH 7.4)

CONJUGATION PROTOCL:

1. Spin down 0.5 mL fluorescent beads (10 mg/mL or 1%) at 14,000xg for 5 min. If high-speed centrifuge is not available, MES buffer could be added to reduce the g force. Longer spin time is also helpful to accommodate lower spin speed.
2. Remove the supernatant and add 0.5 mL MES buffer to resuspend the beads. Sonicate the beads in a bath sonicator to resuspend the beads pellet.
3. Spin down the fluorescent beads at 14,000xg for 5 min.
4. Remove the supernatant and add 0.5 mL MES buffer to resuspend the beads. Sonicate the beads in a bath sonicator to resuspend the beads pellet.
5. Add 35 uL Sulfo-NHS (20 mg/mL) to the bead solution and vortex for 5 seconds.
6. Add 12.5 uL EDC (20 mg/mL) to the bead solution and vortex for 5 seconds.
7. Place the beads solution on a rotator for 15 min.
8. Spin down the fluorescent beads at 14,000xg for 5 min. Optimization of spin speed may be needed because the NHS on the particle may cause aggregation.



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

9. Remove the supernatant and add 0.5 mL Coupling Buffer to resuspend the beads. Sonicate the beads in a bath sonicator to resuspend the beads pellet.
10. Spin down the fluorescent bead at 14,000xg for 5 min and re-suspended the beads in 0.5 mL Coupling Buffer with sonication.
11. Transfer 0.1 mL activated fluorescent beads to a microcentrifuge tube with 0.4 mL Coupling Buffer.
12. Add 50 μ L antibody (1 mg/mL in PBS) to the activated fluorescent beads and vortex for 5 seconds.
13. Place the tube on a rotator for 2 hours.
14. Add 50 μ L quenching buffer (10 mg/mL BSA in 1x PBS) to the fluorescent bead solution.
15. Rotate the solution for 30 min.
16. Spin down the fluorescent bead at 14,000xg for 5 min and re-suspended the beads in 0.5 mL storage buffer with sonication. Optimization of spin speed may be needed because the NHS on the particle may cause aggregation.
17. Spin down the fluorescent bead at 14,000xg for 5 min and re-suspended the beads in 1 mL storage buffer with sonication.

STORAGE:

All the solutions should be stored at 4°C. The pre-weighed EDC vials should be stored at -20°C.

WARRANTIES AND DISCLAIMER

The Ocean Nano Tech MCK products ("Product") is warranted to operate or perform in conformance with published Product specifications at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products **are supplied for research use only**. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current versions of product instructions are available at www.oceannanotech.com. For a faxed copy, call (858) 689-8808.

© 2009 Ocean Nano Tech, LLC. All rights reserved. Unless otherwise indicated, all trademarks are property of Ocean Nano Tech, LLC. Printed in the USA.