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Conjugation Protocol

Red Carboxyl Fluorescent Nanoparticles (F61504)

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: F61504)
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 10,000xg 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 10,000xg 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 10,000xg for 5 min. Optimization of spin speed may be needed because the NHS on the particle may cause aggregation.

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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 10,000xg 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 µL 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 µL 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 10,000xg 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 10,000xg for 5 min and re-suspended the beads in 1 mL storage buffer with sonication.

Storage:

All the solutions should be stored at 2-8°C. The pre-weighed EDC vials should be stored at -20°C.