Conjugation Protocol for Carboxylated Fluorescent Microspheres (F52503)

Introduction

Ocean NanoTech's carboxylated fluorescent microspheres are uniformed polystyrene based microspheres with a high density of carboxyl group on the surface. The microspheres are used to specifically conjugate primary amine-containing proteins or ligands.

Briefly, the fluorescent microspheres are activated using EDC/Sulfo-NHS, and then followed by conjugation to amine groups that are present on the target protein/ligands. The protocol shown below has been used to successfully conjugate bovine serum albumin, streptavidin, and immunoglobulin to Ocean NanoTech's fluorescent microspheres.

Materials Required

- Carboxylated Fluorescent Microspheres (Product ID: F52503, Ocean NanoTech)
- EDC (Product ID: EDC, Ocean NanoTech)
- Sulfo-NHS (Product ID: Sulfo-NHS, Ocean NanoTech)
- Target Protein

Buffer Components

- Activation Buffer: 25 mM MES, 0.01% Tween 20, pH 6.0
- Coupling Buffer: 10 mM PBS, pH 7.4
- Quenching Buffer: 100 mM Tri-HCl, pH 7.4
- Storage Buffer: 10 mM PBS, pH 7.4, 0.1% BSA, 0.02% NaN_3, 0.01% Tween 20

Critical Notes before You Start:

- Any other amine containing molecules (e.g. BSA) in the protein solution, including protein stabilizers, will compete with the conjugation reaction.
- If the targeted protein is already suspended in buffer, such as PBS buffer, this solution could be used directly for conjugation.
- A bath sonicator will be used in this protocol

Protocol

A. Protein Preparation

1. Use ~30 µg proteins per 1 mg microspheres. You may calculate the volume of proteins from the concentration.
2. For example, for 5 mg microspheres, you will need 0.15 mg proteins. Therefore, if the protein concentration is 1 mg/mL, you will need 0.15 mL proteins dissolved in coupling buffer.

$$\frac{0.15\text{ mg protein}}{1\text{ mg/mL (protein concentration)}} = 0.15\text{ mL protein}$$

B. EDC/Sulfo-NHS Preparation

1. Allow the EDC and Sulfo-NHS to come to room temperature before dissolving them.

2. Weigh out 10 mg of EDC, and place it in a microcentrifuge tube. In a separate microcentrifuge tube, weigh out 10 mg of Sulfo-NHS and place it in the tube. Both tubes should be closed tightly until use.
3. Because the EDC solution and Sulfo-NHS solution loses activity with time, each tube is good for one reaction use only and should be prepared only before immediate use. After an aliquot of the EDC and Sulfo-NHS solution is used for one reaction, do not use the remaining EDC and Sulfo-NHS solution in the tube.
4. Add 1.0 mL DI water into each of the pre-weighed EDC tube and Sulfo-NHS tube, yielding a concentration of 10 mg/mL EDC and 10 mg/mL Sulfo-NHS, respectively.
5. Mix well to ensure solids are completely dissolved.

C. Conjugation Procedure

1. Aliquot 0.5 mL of the fluorescent microspheres (1% or 10 mg/mL) into a 1.5 mL microcentrifuge tube. Spin down the fluorescent microspheres at 8,000 rpm (6,800 ×g) for 5 minutes and remove the supernatant.
2. Add 0.5 mL activation buffer in the fluorescent microsphere pellet and re-suspend the fluorescent microsphere by bath sonication for 1 to 3 minutes. Spin down the fluorescent microspheres at 8,000 rpm (6,800 ×g) for 5 minutes and remove the supernatant.
3. Add 0.25 mL activation buffer to the fluorescent microsphere pellet and re-suspend the fluorescent microsphere by bath sonication for 1 to 3 minutes.
4. Add 10 uL mL Sulfo-NHS (10 mg/mL in DI water) and 10uL mL EDC (10 mg/mL in DI water) solution to the fluorescent microspheres solution. (Final concentration of EDC/NHS is 0.4 mg/ml and weight ratio of microsphere to EDC is 50).
5.
6. React at room temperature for 15 minutes with continuous mixing.
7. Spin down the fluorescent microspheres at 8,000 rpm (6,800 ×g) for 5 minutes and remove the supernatant.
8. Add 0.5 mL activation buffer to the fluorescent microspheres pellet and re-suspend the fluorescent microspheres by bath sonication for 1 to 3 minutes.
9. Spin down the fluorescent microspheres at 8,000 rpm (6,800 ×g) for 5 minutes and remove the supernatant.
10. Add 0.4 mL coupling buffer and re-suspend the fluorescent microspheres pellet by bath sonication for 1 to 3 minutes.
11. Add 0.3 mL protein solution (1 mg/mL in coupling buffer) to the fluorescent microsphere. Sonicate the beads/protein mixture with bath sonication for 30 seconds.
12. React at room temperature for 2.5 hours with continuous mixing. Add 0.1 mL quenching buffer and keep mixing the solution for 30 minutes.
13. Spin down the fluorescent microspheres at 8,000 rpm (6,800 ×g) for 5 minutes and remove the supernatant.
14. Add 0.5 mL storage buffer to the fluorescent microspheres pellet and re-suspend the fluorescent microspheres by bath sonication for 1 to 3 minutes.
15. Repeat step #12 and #13 three times to remove the non-conjugated protein in the supernatant.
16. The second resuspension is the purified protein conjugated fluorescent microspheres.