Carboxyl Quantum Dots Conjugation Protocol
Cat# 8CK

Introduction

RayBiotech's carboxyl functionalized Quantum Dots (QDs) are fluorescent nanocrystals with a high density of carboxyl groups on the surface. The QDs can be used to specifically conjugate primary amine containing ligands/protein/antibody with low non-specific binding. The kit contains sufficient reagents and components for 4 conjugation reactions using 0.25 nmoles QDs per reaction.

Briefly, the QDs are activated using EDC (N-ethyl-N'- dimethylaminopropyl-carbodiimide) followed by conjugation to amine groups that are present on the target protein/ligands. The protocol shown below has been used successfully to conjugate bovine serum albumin, streptavidin, and immunoglobulin to RayBiotech's QD nanoparticles.

IMPORTANT: PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING.

Reagents Required

- QDs: 8 µM, 0.125 mL
- EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide), 20 mg. Store at -20 °C upon arrival.
- Activation Buffer, 10 mL (10mM PBS, pH 7.4)
- Quenching Buffer, 1 mL (0.1M tris, pH 7.4)
- Washing/Storage Buffer, 10 mL (10mM PBS, 0.1% BSA, 0.05% NaN3, pH 7.4)

Materials Required

- Pipettes for delivering 10 µL to 1 mL volumes
- Vortex mixer capable of securing 1.5 mL tubes for incubations
- Standard laboratory disposables
- -20 °C freezer and 4°C refrigerator
- 1.7 mL low protein binding centrifuge tubes

Reagents Preparation

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

- Calculation of Protein/Ligand for Conjugation:

We recommend that for 1 nmol QD, 10 nmol protein should be used.

- Protein/Ligand Solution:

Dissolve/dilute protein/ligand in activation buffer or PBS to 1 mg/mL. If your protein/ligand is in amine containing buffer (such as Tris buffer) or at lower concentration, please use spin column to do a buffer exchange (with activation buffer or PBS) and concentrating.

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

- EDC:

Weigh out 2.5 mg EDC and add 250 µL Activation Buffer into the tube. Mix well to dissolve the solids, yielding a final concentration of 10 mg/mL EDC.\

Note: The EDC is not stable in the aqueous solution. Each EDC solution should be prepared only before immediate use and is good for one reaction only. After an aliquot of the EDC solution, do not use the remaining EDC solution.

Conjugation Protocol:

1. In a 1.5 mL micro-centrifuge tube, dilute 125 µL of the 8 µM stock solution of QDs to a concentration of 4 µM by adding 125 µL of Activation Buffer. Thoroughly mix the contents.
2. Divide the mixture into four equal aliquots, placing each into separate micro-centrifuge tubes to prepare four reactions.
3. Add 60 µL of 1 mg/ml protein/ligand solution to the QD solution. Ensure thorough mixing of the components.
4. Add 12.5 µL of EDC solution (10 mg/ml) into the QD protein/ligand solution.
5. React at room temperature for 2 hrs with continuous mixing.
6. Add 10 µL of the Quenching Buffer, ensuring proper mixing. Incubate the mixture at room temperature for 30 minutes.
7. Employ a NanoSep centrifugal device (30K or 100K) or execute ultrahigh-speed centrifugation (as outlined in Table 1) for two rounds to purify the protein-conjugated QD.
8. Resuspend QD conjugates in Washing/Storage buffer to the desired concentration.

Table 1: Suggested ultracentrifugation speed for QD conjugate purifications

QD Wavelength (nm)	Relative centrifugal field for ultracentrifugation (xg)	Time
400	350 k	45-75 minutes
425	350 k	45-75 minutes
450	325 k	45-75 minutes
490	325 k	45-75 minutes
525	325 k	45-75 minutes
540	325 k	45-75 minutes
560	300 k	45-75 minutes
580	275 k	15-45 minutes
600	250 k	15-45 minutes
620	200 k	15-45 minutes
645	125 k	15-45 minutes
665	100 k	15-45 minutes

Storage:

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

Products are supplied for research use only.