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Conjugation of Qdot® ITK™ Amino (PEG) Quantum Dots to Streptavidin

Quantum Dot
invitrogen nanocrystal technologies

Molecular Probes™
invitrogen detection technologies

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PLEASE READ ENTIRE PROTOCOL BEFORE STARTING.

Scope: Conjugation of Qdot® ITK™ Amino (PEG) Quantum Dots to streptavidin and purification from excess streptavidin.

Required materials:

2 nmol Qdot® ITK™ Amino (PEG) Quantum Dots in borate buffer (50 mM borate, pH 8.3)
1 mg *Bis*[sulfosuccinimidyl] suberate (BS3: Pierce catalog #21580)
4.8 mg of Streptavidin (BioSource) in 10 mg/ml solution (in DI water)
(or equivalent)
100KD ultrafiltration units:
4mL: Amicon Ultra-4 —Millipore Corp. Cat # UFC810008
15 mL: Amicon Ultra 15—Millipore Corp. Cat # UFC910008
Desalting Columns: NAP-5 columns (Amersham Biosciences 17-0853-01)
Filters: Acrodisc 25 mm PF Syringe Filter w/0.8/0.2 uM Supor Membrane or
Acrodisc Syringe Filter 0.2 uM Supor Membrane Low Protein Binding Non Pyrogenic
PBS: Phosphate buffered saline, pH 7.4 (Sigma Cat #P-3813)
Quenching buffer: (optional) 1M Glycine, pH 6.5 (in DI water)

Procedure:

Transfer 2 nmol Qdot® ITKTM Amino (PEG) quantum dots into 100 kD ultrafiltration unit. Fill remaining volume of ultrafiltration unit with 1X PBS, pH 7.4. Centrifuge according to manufacturers instructions. Refill unit with 1X PBS, pH 7.4 and centrifuge again according to manufacturers instructions until volume is reduced to near the initial volume (~ 250 uL).

1. Transfer quantum dots to glass vial or siliconized eppendorf tube. Add BS3 to Qdot® nanocrystal solution such that your final ratio of QD to BS3 is 8-10 μM dots/1 mM BS3. *If the volume of quantum dots is 250 uL then the concentration is 8 uM (2 nmoles/0.25 mL).* Let react for 1/2 hour at room temperature on rotator. This can be in a glass vial or in a siliconized eppendorf tube.
2. Equilibrate a Nap column with 1X PBS, pH 7.4 according to manufacturer's instructions (5 complete buffer exchanges). Purify the quantum dots from excess cross-linker by buffer exchange on a pre-equilibrated Nap 5 (or 10 or 25) column to 1X PBS, pH 7.4, according to the manufacturer's instructions.
3. Collect the colored eluent (use of a hand-held UV lamp may help) into a glass vial or siliconized eppendorf tube containing a 40-fold excess of streptavidin (10-12 mg/mL stock). Mix gently and let react 2 hours. Quench with 1 M glycine by adding glycine to a final concentration of approximately 50 mM and let react for 15 minutes.
4. Purify the conjugate from excess streptavidin by ultrafiltration (100kD) into 50 mM borate, pH 8.3 according to manufacturer instructions. This typically takes 5 or 6 rounds. Gel filtration (Superdex 200 or equivalent) generally achieves better separation from the excess biomolecule.
5. Filter material through a 0.2 μm syringe filter or a 0.8/0.2 μm combination filter. Store at 4° C