invitrogen™

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

Quantum Dot
invitrogen nanocrystal technologies

Molecular Probes™
invitrogen detection technologies

P/N 90-0152, Rev 1

mp19028 12-Dec-2005

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

PLEASE READ ENTIRE PROTOCOL BEFORE STARTING.

Materials

2 nmol Qdot ITK Amino (PEG) Quantum Dots in borate buffer (50 mM borate, pH 8.3)
0.3 mg *Bis*[sulfosuccinimidyl] suberate (BS3: Pierce Chemicals, Cat #21580)
150 μg of Amino Phalloidin, Hydrochloride (Alexis Biochemicals, Cat # ALX-350-266)

CAUTION: Phalloidin is **TOXIC**. Wear gloves and change them frequently. Use a needle to inject DI water into sealed vial containing solid phalloidin to prepare a 2 mg/ml solution. **DO NOT OPEN** the vial before dissolving Phalloidin. Perform the conjugation in one location and clean thoroughly at the end of the procedure.

100 kDa ultrafiltration units:
4 mL: Amicon Ultra 4 (Millipore Corp. Cat # UFC810008)
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 ITK Amino (PEG) quantum dots into 100 kDa 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 μL).

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.
2. Equilibrate a NAP-5 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 column to 1X PBS, pH 7.4, according to the manufacturer's instructions. **Do not collect more solution from the column than specified in the instructions.**
3. Collect the colored eluent into a glass vial or siliconized eppendorf tube containing a 40-fold excess of Phalloidin (typically 36 uL of a 2 mg/mL stock for a 2 nmol scale conjugation). Mix gently and let react 2 hours. Quench with 1 M glycine by adding glycine to a final concentration of 50 mM and let react for 15 minutes.
4. Purify the conjugate from excess phalloidin by ultrafiltration (100 kDa) 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.