

## Conjugation of Qdot® ITK™ Carboxyl Quantum Dots to Streptavidin

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**PLEASE READ ENTIRE PROTOCOL BEFORE STARTING.**

**Scope:** Conjugation of Qdot® ITK™ Carboxyl Quantum Dots to streptavidin and purification from excess streptavidin.

### **Required materials:**

2 nmol Qdot® ITK™ Carboxyl Quantum Dots (in 50 mM sodium borate, pH8.3)

10 mg N-ethyl-N'-dimethylaminopropyl-carbodiimide (EDC)

4.8 mg of Streptavidin (BioSource) in 10 mg/ml solution (in 10 mM Borate buffer)  
(or equivalent)

100KD ultrafiltration units:

4mL: Amicon Ultra-4 —Millipore Corp. Cat # UFC810008

15 mL: Amicon Ultra 15—Millipore Corp. Cat # UFC910008

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

### **Procedure:**

QDs: ~2 nmol (250 µL at 8 µM concentration)

1 equivalent

Streptavidin: 2-80 nmol (0.12 mg-4.8 mg at 10 mg/mL)

1-40 equivalent

EDC (3000 nmol) ( 0.57 mg at 10 mg/mL)

1500 equivalent

1. In a small glass vial with a small stirbar, dilute the stock of dots to 1 µM using 10 mM borate buffer, pH 7.4.
2. Add streptavidin to this solution by pipetting in the appropriate amount from a ~10 mg/ml stock solution.
3. Weigh out ~5 mg of EDC in a 1.7 ml eppendorf tube. Dilute to 10 mg/ml (e.g. 5 mg weighed diluted in 0.5 ml H<sub>2</sub>O) in H<sub>2</sub>O and immediately add 57 µL of this stock solution to the stirred quantum dot solution.
4. Reduce stirring speed and stir gently for 1-2 hours.
5. Filter the conjugate solution through a 0.2 um PES membrane unit and transfer to a clean centrifugal ultrafiltration unit.
6. Spin in a benchtop centrifuge according to manufacturer instructions for at least 5 buffer exchanges with 50 mM borate buffer, pH 8.3, ensuring each time that volume concentration is > 10 fold (e.g. 4 mL to <400 µL). Note: for large excess of protein, additional concentrations may be required.
7. Filter material through a 0.2 µm syringe filter or a 0.8/0.2 µm combination filter. Store at 4° C