Carboxyl QDs conjugation protocol

Material provided	Storage
• Quantum dots nanoparticles with carboxyl (-COOH) functionalisation, 1 mL at a concentration of 2 uM.	• Store at 2–8 °C. • Do not freeze! • Protect from light.

Material NOT provided
• Antibody solution at 1 mg/mL in a suitable buffer (minimum antibody concentration: 0.5 mg/mL), such as Dubecco's PBS (DPBS) and HEPES. *Amine-containing buffers such as Tris are not suitable for this reaction!* • 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). • N-hydroxysuccinimide (NHS) or N-hydroxysulfosuccinimide (sulfo-NHS). • Glycine (for quenching). • Centrifugal filter with a MWCO of 150 kDa, such the Amicon® Ultra 0.5 mL (Merck Millipore).

Procedure to conjugate 100 µg of antibody to QD-COOH

Step 1 – QDs activation

1. In a 1.5 mL tube, add 140 µL of carboxyl QDs to 140 µL of DPBS.
2. Freshly prepare a 10 mg/mL solution of EDC and a 12 mg/mL solution of NHS, both in ultrapure water.
3. Immediately add 10 µL of the EDC solution to the QDs solution, followed quickly by 10 µL of the NHS solution.
4. Mix the QDs at room temperature for 20 minutes.

Step 2 – QDs + antibody reaction

1. Purify the activated QDs from excess EDC and NHS via spin filtration, repeating the filtration three times and resuspending the nanoparticles in DPBS after each step.
   NOTE: it is important to complete this step as quickly as possible, as activated QDs will hydrolyse and lose their reactivity.
2. Collect the purified QDs, preferably in a 200 µL tube. Adjust the final volume to 100 µL using DPBS.

3. Add 100 µL of the antibody solution to the QDs, and mix for at least 3 hours at 4 °C (overnight incubation is also acceptable).
4. Quench any unreacted NHS groups by adding 15 µL of 50 mM glycine.
5. Store the QD–antibody conjugate at 2–8 °C, protected from light. Do not freeze! The conjugate should remain stable for at least 1 month.

NOTE: To conjugate a smaller amount of protein, take an aliquot of the carboxyl-functionalised QDs and place it in a new tube. Maintain the same molar ratios between EDC, NHS, antibody, buffer, and QDs.

NOTE: Removal of excess antibody can be achieved via size exclusion chromatography or centrifugal filtration with a MWCO > 150 kDa.