Azide QDs conjugation protocol

Material provided	Storage
• Quantum dots nanoparticles with azide (-N_3) functionalisation, 500 µL at a concentration of 2 uM. • Borate Buffered Saline (BBS) buffer, 10 mM, pH 8.4, 8 g/L NaCl • Linker solution (0.1 mM)	• 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 BBS, DPBS, and HEPES. *Amine-containing buffers such as Tris are not suitable for this reaction!* • Dubecco's PBS (DPBS). • Glycine (for quenching). • Centrifugal filter with a maximum MWCO of 50 kDa, such the Amicon® Ultra 0.5 mL (Merck Millipore).

Procedure to conjugate 100 µg of antibody to QD-N_3

Day 1 – Linker + antibody reaction

1. In a 1.5 mL tube, add 100 µL of antibody (1 mg/ml) to 500 µL of BBS.
2. Immediately add 12 µL of the linker solution to the tube containing the antibody solution and mix for 18 hours at 4 °C.
   (*These quantities give the preferred molar ratio of linker to antibody of 2:1.*)

Day 2 – Linker-antibody + QDs reaction

1. Quench the linker reaction with 15 µL of 50 mM glycine.
2. Purify the antibody from excess linker via spin filtration three times, using DPBS to resuspend the protein after each step.
3. Collect the concentrated protein, preferably in a 200 µL tube. Add enough DPBS to adjust the final volume to 100 uL.
4. Add 140 µL of QDs to the antibody-linker solution, and mix for 3 hours at 4 °C.
5. After 3 hours, the solution is ready to use.

6. 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 azide-functionalised QDs and place it in a new tube. Maintain the same molar ratios between the linker solution, antibody, buffer, and QDs. If the scale of the reaction requires less than 1 µL of linker, dilute the linker 1:10 in DMSO (final concentration 0.01 mM), and immediately use the diluted solution for the antibody modification.