



## Conjugation Protocol of Carboxyl Quantum Dot Beads by Carbodiimide Chemistry

### Material:

1. Carboxyl Quantum Dot Beads, 0.2 mL;
2. 0.02 M MES buffer (2-(4-morpholino) ethanesulphonic acid buffer), which was adjusted to pH 6;
3. EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride), 20 mg/mL in 10 mM MES-NaOH buffer (pH = 6);
4. NHS (N-hydroxysuccinimide), 20 mg/mL in 10 mM MES-NaOH buffer (pH = 6);
5. 80 µg protein or antibody;
6. 10 mM MES-NaOH buffer (pH = 6);
7. Blocking Buffer: 100 mM glycine buffer with 1% BSA.

### Procedure:

1. Transfer the carboxyl Quantum Dot Beads suspension (0.2 mL, 1 µmol/L) into a 0.2 ml 0.02 M MES buffer (pH 6);
2. Then, add 4 µL 20 mg/mL EDC-HCl and 4 µL 20 mg/mL NHS, incubate the suspension with continuous shaking for 15 min at 37 °C.
3. Centrifuge at 8000x g for 20 minutes. Then, remove the supernatant carefully.
4. Resuspend the pellet in 0.2 mL of 10 mM MES-NaOH buffer (pH = 6).
5. Add 80 µg protein or antibody - incubate the suspension with continuous mixing for 1 hours at 37 °C;
6. Add 20 µl Blocking Buffer - incubate the suspension with continuous mixing for 30 min at 37 °C;
7. Centrifuge at 8000x g for 15 minutes. Then, remove the supernatant carefully.
8. Resuspend the Beads in PBS-buffer (pH = 7.4);
9. Stabilize the suspension by addition of BSA if necessary.

### Note:

*Low ionic strength is beneficial to colloid stability.*

*Use EDC-HCl and NHS buffer right after they were ready.*

*This protocol is intended to provide general guidelines for the binding of biomolecules or related compounds. Further optimization may be required in order to achieve optimal functionality and stability from case to case.*