

Table 1. Summary of quantum dot labeling protocol for neurons and glia

Pre-processing and Fixing

Remove media from wells by gently aspirating.

Wash cells with warmed PBS.

Fix cells with 4% paraformaldehyde (Electron Microscopy Sciences, catalog #157 15-S) in PBS for 10 minutes at room temperature.

Wash cells 3X with PBS.

Permeabilize cells with 0.2% Triton X-100 (Fisher Scientific, catalog #BP151-100) in PBS for 5 minutes.

Wash cells 3X for 5 minutes with PBS.

Incubate with 10% Horse Serum in PBS for 30 minutes at room temperature.

Rinse with PBS.

Apply Streptavidin/Biotin blocking kit (Vector Labs, catalog #SP-2002).

Primary Incubation

Rinse with PBS.

Add biotinylated molecule of interest. (E.g. antibodies; use ProtOn Biotin Labeling Kit or similar for biotinylation; Vector Labs, catalog #PLK-1202).

Incubate 2 hours at room temperature.

[Biotinylated secondary antibody for one hour- alternative 3 step labeling protocol]

Remove antibodies by gently aspiration and rinse 3X with PBS.

Quantum dot incubation

Add Streptavidin conjugated quantum dots (We used Quantum Dot Corporation's 605 nm quantum dots here, catalog #1010-1) in 10% Horse Serum.

Incubate 1 hour at room temperature.

Rinse 3X with PBS.

Mount with 90% glycerol (Sigma, catalog #G-6279) in PBS.