Immunofluorescent staining for neuronal marker MAP2

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We use this protocol and it's working

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ABSTRACT

This is the protocol for immunofluorescent staining for neuronal marker MAP2.

1 1. Treat differentiated SH-SY5Y cells with 40 ug/mL eumelanin or pheomelanin or PBS for 24 hours.

2 2. Process cells using Cytofix/Cytoperm™ fixation/permeabilization solution (BD554714, Thermo Fisher Scientific) and block with 5% normal goat serum.

3 3. Add primary antibody against microtubule-associated protein 2 (MAP2)(30 µg/mL, OSM00036G, Thermo Fisher Scientific) and incubate at 4°C overnight.

4 4. Add secondary antibody (1:1000, Alexa 594-conjugated, A11012, Thermo Fisher Scientific) and incubate for 2 hours at room temperature.

5 5. Stain nuclei with DAPI.

6 6. For MAP2-positive cell counting, three images are captured randomly from each well using FluoView FV300 confocal microscope under a 60x objective lens. MAP2 and DAPI channels are merged, and MAP2-positive cells in each random visual field of 0.045000 mm2 are counted using ImageJ.