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Immunofluorescence Staining and Analysis of Astrocyte-Neuron Co-Cultures

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We use this protocol and it's working

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Abstract

Immunofluorescence Staining and Analysis of Astrocyte-Neuron Co-Cultures

1 **Fixation**

1.1 - Fix astrocyte-neuron co-cultures on glass coverslips on Day in Vitro 12 (DIV 12) with warm 4% paraformaldehyde (PFA) for 7 minutes.

1.2 - Wash coverslips 3 times with phosphate-buffered saline (PBS).

2 **Blocking**

2.1 - Block coverslips in a blocking buffer containing 50% normal goat serum (NGS) and 0.4% Triton X-100 for 30 minutes at room temperature.

2.2 - Wash coverslips with PBS.

3 **Primary Antibody Incubation**

3.1 - Incubate samples overnight at 4°C in primary antibodies diluted in blocking buffer containing 10% NGS.

3.2 - Incubate coverslips in Alexa Fluor conjugated secondary antibodies (Life Technologies) for 2 hours at room temperature.

3.3 - Wash coverslips again with PBS.

4 **Mounting**

4.1 - Mount coverslips onto glass slides (VWR Scientific) using Vectashield mounting media containing DAPI (Vector Labs).

4.2 - Seal coverslips with nail polish.

5 **Imaging**

5.1 - Image coverslips using an AxioImager M1 fluorescence microscope (Zeiss) at 40x magnification in red, green, and/or DAPI channels using a CCD camera.

6 **Morphological Analysis**

6.1 - Analyze astrocyte morphological complexity using FIJI with the Sholl analysis plugin (https://github.com/Eroglu-Lab/In-Vitro-Sholl).

6.2 - Ensure analysis is performed on healthy astrocytes with strong expression of fluorescent markers and single, non-overlapping nuclei (DAPI stain).

7 **Statistical Analysis**

7.1 - Conduct statistical analyses using custom code in R (https://github.com/Eroglu-Lab/In-Vitro-Sholl).

7.2 - Use a mixed-effect model with Tukey post-test for Sholl analysis to evaluate differences between experimental conditions, treating variability per experiment as a random effect.

8 **Quality Control**

8.1 - Verify the health of astrocyte-neuron co-cultures by ensuring the peak number of astrocyte intersections is ≥ 25 in the control condition.

8.2 - Document the exact number of independent experiments and cells analyzed in the figure legend for each experiment.

9 Notes:

9.1 - Maintain sterility and avoid contamination during all steps.

9.2 - Ensure imaging settings are consistent across experimental conditions for accurate comparison.

9.3 - Validate antibody specificity and optimize staining conditions for reproducibility.