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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.