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Immunofluorescence staining for postmortem mouse brain tissue

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Elizabeth Phelan[1]

[1]Northwestern University, Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815

E Elizabeth Phelan
Northwestern University

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We use this protocol and it's working

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Abstract

This is a basic protocol for staining mouse brain tissues using immunofluorescence and immunohistochemistry techniques.

Troubleshooting

Preparation

1 For this protocol I recommend staining slices in a 12-well plate, and washing the slices in a separate 24-well plate. Each well can hold one solution or wash for the slices (about 1mL of solution in each well should be fine). Be sure to label the plate with the contents of each well.

2 Prepare the blocking solution ahead of time: 5% Normal Donkey Serum in PBS1x with 0.3% TritonX. For 10mL of solution, add 9.5 mL of the PBS + TritonX solution and 0.5mL of NDS.

3 The antibody solutions can also be prepared ahead of time, they are created by simply adding the antibodies to aliquots of the blocking solution prepared above. The concentration of the primary and secondary antibody solutions will vary depending on the specific antibody and desired target of the staining; a solution of 1:250 or 4uL/mL concentration is recommended for first-time antibody stains.

Staining

4 Wash the tissue slices in 1x PBS for ten minutes at room temperature for 3 total washes.

5 Place the tissue slices in blocking solution for 30 minutes on the shaker at room temperature.

6 Move the tissue slices to the primary antibody solution and leave to shake gently overnight at 4 degrees celsius.

7 Wash the slices in 1x PBS plus 0.05% Tween, 4 times for 5 minutes at room temperature.

8 Place the tissue slices in the fluorescent secondary antibody solution, shake gently for 2 hours at room temperature, making sure to cover the slices and their solution to avoid bleaching the fluorescence.

9 Wash the slices in 1x PBS plus 0.05% Tween, 4 times for 5 minutes at room temperature.

10 Wash the slices one final time in 1x PBS for 5 minutes at room temperature.

11 Mount the tissues immediately.