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Version 2

Immunostaining of Human Frontal Cortex Sections V.2

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We use this protocol and it's working

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Abstract

Immunostaining of Human Frontal Cortex Sections

Attachments



Supplemental Table 1...

15KB

1 ****Tissue Acquisition****

- 1.1 - Obtain 40 µm thick floating sections of human frontal cortex from Banner Sun Health Research Institute in Sun City, Arizona.
- 1.2 - Use sections from 4 control subjects and 3 LRRK2 G2019S mutation carrier subjects.

2 ****Subject Information****

- 2.1 - Verify that control subjects had no history of dementia, neurological, or psychiatric disorders at the time of death (Refer to Supplemental Table 1).
- 2.2 - Ensure that informed and written consent was obtained from all donors.

3 ****Preparation of Sections****

- 3.1 - Wash sections in 1x TBS containing 0.3% Triton X-100 (TBST) to remove any residual fixative and debris.

4 ****Blocking****

- 4.1 - Block non-specific binding by incubating sections in 3% normal goat serum (NGS) diluted in TBST for blocking, typically for 1 hour at room temperature.

5 ****Primary Antibody Incubation****

- 5.1 - Incubate sections overnight at 4°C with gentle shaking in primary antibodies diluted in blocking buffer: GFAP (chicken, 1:250; AB5541, Millipore Sigma), Phospho-ERM (Rabbit, 1:250; #3726, Cell Signaling), S100β (Mouse IgG1, 1:200; S2532, Millipore Sigma), and MAP2 (Mouse IgG1, 1:200; 13-1500, Thermo Fisher Scientific).

6 ****Washing****



6.1 - Wash sections thoroughly with TBST to remove unbound primary antibodies.

7 ****Secondary Antibody Incubation****

7.1 - Incubate sections with Alexa Fluor conjugated secondary antibodies (diluted 1:200 in TBST) for 2-3 hours at room temperature in the dark.

8 ****Final Washing****

8.1 - Wash sections again with TBST to remove unbound secondary antibodies.

9 ****Mounting****

9.1 - Mount sections onto glass slides using a homemade mounting media composed of: 90% Glycerol, 20 mM Tris pH 8.0, 0.5% n-Propyl gallate

10 ****Sealing****

10.1 - Seal coverslips with nail polish to prevent drying and movement during imaging.