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Immunostaining of Human Frontal Cortex Sections

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We use this protocol and it's working

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Abstract

Immunostaining of Human Frontal Cortex Sections

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)

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.