

Jul 11, 2024

Version 1

Immunostaining of Human Frontal Cortex Sections V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.j8nlk8mk5l5r/v1>

Shiyi Wang¹

¹Duke University



Shiyi Wang

Duke University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.j8nlk8mk5l5r/v1>

Protocol Citation: Shiyi Wang 2024. Immunostaining of Human Frontal Cortex Sections. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.j8nlk8mk5l5r/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: July 11, 2024

Last Modified: July 11, 2024

Protocol Integer ID: 103201

Keywords: ASAPCRN, human frontal cortex sections immunostaining, immunostaining, cortex, human frontal cortex section

Funders Acknowledgements:

Aligning Science Across Parkinson's (ASAP) initiative

Grant ID: ASAP-020607

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

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.