

Jul 10, 2024



Human brain section staining

DOI

dx.doi.org/10.17504/protocols.io.6qpvr4bb2gmk/v1

Shiyi Wang¹

¹Duke University

ASAP Collaborative Rese...



Shiyi Wang

Duke University

OPEN ACCESS



DOI: dx.doi.org/10.17504/protocols.io.6qpvr4bb2gmk/v1

Document Citation: Shiyi Wang 2024. Human brain section staining. protocols.io

https://dx.doi.org/10.17504/protocols.io.6qpvr4bb2gmk/v1

License: This is an open access document 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

Created: May 23, 2023

Last Modified: July 10, 2024

Document Integer ID: 82334

Keywords: ASAPCRN

Funders Acknowledgement: Aligning Science Across Parkinson's (ASAP) initiative Grant ID: ASAP-020607

Abstract

How to stain Human brain sections





- 1. Floating human frontal cortex sections of 40 µm thickness were obtained from Banner Sun Health Research Institute in Sun City, Arizona (4 control and 3 LRRK2 G2019S mutation carrier subjects).
- None of the control subjects had a history of dementia or neurological or psychiatric disorders at the time of death (See Supplemental Table 1). Informed and written consent was obtained from the donors.
- 2. For immunostaining, sections were washed in 1× TBS containing 0.3% Triton X-100 (TBST), blocked in 3% NGS diluted in TBST, and incubated in primary antibody 2-3 nights at 4°C with shaking.
- 3. Primary antibodies used were GFAP (chicken, 1:250; AB5541, Millipore Sigma) and phospho-ERM (Rabbit, 1:250; #3726, Cell Signaling).
- 4. Following primary incubation, sections were washed in TBST, incubated in Alexa Fluor conjugated secondary antibodies diluted 1:200 (Life Technologies) for 2-3 hours at room temperature, washed with TBST, and mounted onto glass slides using a homemade mounting media (90% Glycerol, 20 mM Tris pH 8.0, 0.5% n-Propyl gallate) and sealed with nail polish.