Jul 11, 2024

Immunohistochemistry on mouse brain sections

DOI
dx.doi.org/10.17504/protocols.io.j8nlk8m4xl5r/v1

Shiyi Wang[1]

[1]Duke University

ASAP Collaborative Rese…

Shiyi Wang
Duke University

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.j8nlk8m4xl5r/v1

Protocol Citation: Shiyi Wang 2024. Immunohistochemistry on mouse brain sections. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlk8m4xl5r/v1

Protocol status: Working
We use this protocol and it's working

Created: July 11, 2024

Last Modified: July 11, 2024

Protocol Integer ID: 103197

Keywords: ASAPCRN

Funders Acknowledgement:
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and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind.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

Immunohistochemistry on mouse brain sections

1 **Anesthesia and Perfusion**

1.1 - Anesthetize mice with 200 mg/kg Avertin.

1.2 - Perform transcardial perfusion using TBS/Heparin followed by 4% paraformaldehyde (PFA).

2 **Brain Collection and Post-Fixation**

2.1 - Remove the brain and post-fix it in 4% PFA overnight at 4°C.

3 **Cryoprotection and Storage**

3.1 - Cryoprotect the brain in 30% sucrose solution until sunk.

3.2 - Embed the brain in a solution containing 2 parts 30% sucrose and 1 part O.C.T. (TissueTek).

3.3 - Store the brain blocks at −80°C until sectioning.

4 **Sectioning**

4.1 - Section the brain into 30 µm, 40 µm, or 100 µm thick coronal slices using a cryostat.

4.2 - Store the sections in a 1:1 mixture of TBS and glycerol at −20°C for further use.

5 **Preparation of Sections**

5.1 - Wash the brain sections in 1x TBS containing 0.2% Triton X-100 (TBST).

6 **Blocking**

6.1 - Block non-specific binding by incubating sections in 10% normal goat serum (NGS) diluted in TBST for 1 hour at room temperature.

7 **Primary Antibody Incubation**

7.1 - Incubate sections with primary antibodies diluted in blocking buffer (10% NGS in TBST) for 2-3 nights at 4°C with gentle shaking.

7.2 - Primary antibodies used: - Anti-LRRK2 (Rabbit, 1:500; ab133474, Abcam), HA (Rat, 1:500; 11867423001, Roche), Phospho-ERM (Rabbit, 1:500; 3141, Cell Signaling), Sox9 (Rabbit, 1:500; AB5535, Millipore), GFAP (Rabbit, 1:500; Z0334, Agilent DAKO), VGluT1 (Guinea pig, 1:2000; 135304, Synaptic Systems), PSD95 (Rabbit, 1:300; 51-6900, Innovative Research), VGAT (Guinea pig, 1:1000; 131004, Synaptic Systems), Gephyrin (Rabbit, 1:1000; 147011, Synaptic Systems)

8 **Secondary Antibody Incubation**

8.1 - Wash sections with TBST.

8.2 - Incubate sections in Alexa Fluor conjugated secondary antibodies diluted 1:200 in TBST for 2-3 hours at room temperature.

9 **Mounting**

9.1 - Wash sections in TBST.

9.2 - Mount sections onto glass slides using a homemade mounting media (90% Glycerol, 20 mM Tris pH 8.0, 0.5% n-Propyl gallate).

9.3 - Seal the edges of the coverslip with nail polish.

10 **DAPI Staining**

10.1 - Add DAPI (1:50,000) to the secondary antibody solution for the final 10 minutes of incubation.

11 **Imaging**

11.1 - Acquire images using a fluorescence microscope (e.g., Olympus FV 3000).