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Immunohistochemistry on mouse brain sections V.2

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We use this protocol and it's working

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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), S100 β (Mouse IgG1, 1:200; S2532, Millipore Sigma), MAP2 (Mouse IgG1, 1:200; 13-1500, Thermo Fisher Scientific), and Iba1 (Chicken, 1:500; 234009, 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).