IMMUNOHISTOCHEMISTRY (Brain Sections)

Reference

1. Lang, A. E., Riherd Methner, D. N., & Ferreira, A. (2014). Neuronal degeneration, synaptic defects, and behavioral abnormalities in tau_{45-230} transgenic mice. Neuroscience, 275, 322–339. https://doi.org/10.1016/j.neuroscience.2014.06.017

Solutions

1. Fixative: 4% Paraformaldehyde prepared in PBS, pH 7.4 w/ NaOH
2. Blocking Buffer: 5% Bovine Serum Albumin prepared in PBS

Abbreviations

1. PFH: Paraformaldehyde
2. IP injection: Intraperitoneal injection
3. RT: Room Temperature
4. PBS: Phosphate Buffered Saline
5. O.C.T.: Optimal Cutting Temperature
6. BSA: Bovine Serum Albumin

Protocol

Transcardial Perfusion

1. Transcardially fix brain with 4% PFH following protocols approved by the Institution Animal Care and Use Committee in accordance with United States Public Health Service regulations and applicable deferral and local laws before performing any experiments using vertebrate animals.

Brain Slicing

1. Place tissue in block as desired, fill block with O.C.T./embedding medium and place it on dry ice until frozen.
2. Cut 15-20 µM-thick slices with the cryostat and mount them on gelatin-coated slides.
3. Store the slides at -20°C.

Immunohistocytochemistry

4. Staining: Rinse slides 3 x 5 min with PBS.
5. Block in 5% BSA or Normal Goat Serum and 0.1% Triton prepared in PBS for 2 hrs at RT or at 4°C overnight.
6. Incubate with primary antibody plus 2% BSA and 0.05% Triton for 3 hrs at RT or at 4°C overnight.
7. Rinse slides 6 x 5 minutes with PBS.
8. Incubate with secondary antibody plus 2% BSA for 1 hr at RT or at 37°C.
9. Rinse slides 3 x 5 minutes and mount with mounting medium.
10. Let slides dry overnight at RT.