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# Immunohistochemistry

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ABSTRACT

This protocol describes immunohistochemical staining of fixed brain sections.

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Immunohistochemisty, IHC, Immunostaining, Brain, Mouse, ASAPCRN



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## 1 Sectioning

1.1 Using freezing microtome section brain at 30-35 µm

1.2 Place sections in PBS in a 24-well plate until ready for immunohistochemistry; if not performing immunohistochemistry that day, cover 24 well plate and place at 4ºC overnight

2 Blocking.
Incubate sections in blocking buffer at **Room temperature** for 1-2 hours.
Blocking buffer is: 3% NDS (normal donkey serum)/ 0.1% triton (also called NDST) in PBS.

3 Primary Antibody.
Prepare primary antibody in 3% NDS at desired concentration (make sure this is the final concentration in the well if you are adding more than one primary) and let sections sit on shaker **Overnight** at **4 °C**

4 Wash.
Wash sections 3-5 times in PBS for 10-15 minutes each at **Room temperature**

5 Secondary Antibody.
Prepare secondary antibody in 3% NDS at desired concentration and let sections sit on shaker for 2-4 hours at **4 °C**

1:500 is default concentration for secondary antibodies.

6 Wash.
Wash sections 3-5 times in PBS for 10-15 minutes each at **Room temperature**

7 Mounting.
Mount sections onto slides with Vectashield hardset mounting medium (with or without DAPI).

8 Imaging.



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8.1 For low-magnification display of viral expression patterns or tyrosine hydroxylase staining, we typically use 10X stitched epifluorescence images.

8.2 For evaluation of individual neurons and overlap of fluorescent markers, we typically use 40X epifluorescence or confocal Z-stacks.



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