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Alexandra Nelson¹

¹University of California San Francisco



Kelsey Barcomb

CU Anschutz

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We use this protocol and it's working

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Abstract

This protocol describes immunohistochemical staining of fixed brain sections.



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 4°C 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

Note

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.

- 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.