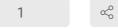


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## Immunofluorescent Staining

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This protocol is used to stain cryosectioned mouse brain tissue.

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- To cryo-sectioned brain tissue, wash with 1X phosphate buffered saline (PBS) for 3x 5-minute washes.
- 2 Incubate in blocking buffer for 1 hour at room temperature. Blocking buffer: 10% serum + 0.5% Triton X-100 in 1X PBS

1h

3 Wash tissue with 1X PBS.

5m

4 Incubate in primary antibody diluted in blocking buffer overnight at 4°C.

1d

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5	Wash tissue with 1X PBS for 5x 5-minute washes.	30m
6	Incubate in secondary antibody diluted in blocking buffer for 1 hour at room temperature	1h e.
7	Wash tissue with 1X PBS for 5x 5-minute washes.	30m
8	If tissue wasn't previously mounted on a slide, mount on a superfrost plus slide and let d room temperature for at least 10 minutes.	15m ry at
9	Coverslip with fluorescent mounting medium and a #1.5 coverslip. Outline the coverslip	1m with

clear nailpolish.