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Immunofluorescent Staining

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol is used to stain cryosectioned mouse brain tissue.

1	To cryo-sectioned brain tissue, wash with 1X phosphate buffered saline (PBS) for 3×5 -minute washes.	15m
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
5	Wash tissue with 1X PBS for 5× 5-minute washes.	30m
6	Incubate in secondary antibody diluted in blocking buffer for 1 hour at room temperature.	1h
7	Wash tissue with 1X PBS for 5× 5-minute washes.	30m
8	If tissue wasn't previously mounted on a slide, mount on a superfrost plus slide and let dry at room temperature for at least 10 minutes.	15m
9	Coverslip with fluorescent mounting medium and a #1.5 coverslip. Outline the coverslip with clear nailpolish.	1m