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Immunofluorescent Staining

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Haley Geertsma[1]

[1]University of Ottawa

Haley Geertsma
University of Ottawa

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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^{O}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