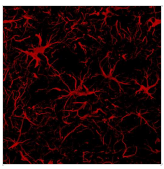

Feb 16, 2021

Immunofluorescence staining of PFA or fresh frozen mouse brain section

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1 *Works for me* This protocol is published without a DOI.

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SUBMIT TO PLOS ONE

ABSTRACT

This protocol is suitable for immunofluorescence staining of PFA fixed or Fresh Frozen mouse brain sections.

PROTOCOL CITATION

Daniel Manrique-Castano 2021. Immunofluorescence staining of PFA or fresh frozen mouse brain section. **protocols.io** https://protocols.io/view/immunofluorescence-staining-of-pfa-or-fresh-frozen-bsf6nbre

CREATED

Feb 16, 2021

LAST MODIFIED

Feb 16, 2021

PROTOCOL INTEGER ID

47326

MATERIALS TEXT

Blocking-Permeabilization buffer

1X PBS (Phosphate-buffered saline) **Contributed by users**

[M]**0.1 % (v/v)** Triton-X100 **Contributed by users**

Tween 20 **Bio-rad**

[M]**0.05 % (v/v)** **Laboratories Catalog #170-6606-MSDS**

Glycine **Sigma –**

[M]**0.3 Molarity (M)** **Aldrich Catalog #G8898**

Primary antibody buffer

1X PBS (Phosphate-buffered saline) **Contributed by users**

Tween 20 **Bio-rad**

[M]**0.1 % (v/v)** **Laboratories Catalog #170-6606-MSDS**

Normal Goat Serum **Gibco - Thermo**

[M]**5 % (v/v)** **Fischer Catalog #LSPCN5000** or

Normal Donkey Serum **Contributed by**

users Catalog #017-000-121 depending on your secondary antibody species.

Secondary antibody buffer

1X PBS (Phosphate-buffered saline) **Contributed by users**

Normal Goat Serum **Gibco - Thermo**

[M]**1 % (v/v)** **Fischer Catalog #LSPCN5000**

Normal Goat Serum **Gibco - Thermo**

[M]**1 % (v/v)** **Fischer Catalog #LSPCN5000** or

Normal Donkey Serum **Contributed by**

users Catalog #017-000-121 depending on your secondary antibody species.

SAFETY WARNINGS

Paraformaldehyde (PFA) and DAPI are highly toxic substances. Manipulation should be performed carefully and according to security measurements.

BEFORE STARTING

Please read the whole protocol before starting the procedure.

Rehydration and permeabilization 1h 10m

1 If fixed sections are kept in **-80 °C** , rehydrate them before starting the staining procedure by incubating them in **PBS** for **00:10:00** . 10m

For fresh frozen sections kept at **-80 °C** , incubate with [M]**4 % volume** PFA during **00:15:00** followed by 3 washes in **PBS,** **00:05:00** each.

2 To permeabilize and block for unspecific staining, decant the PBS and incubate the section with **Blocking-Permeabilization buffer** for **01:00:00** at **Room temperature** in al humid chamber/box to prevent sections from drying out. 1h

If required, encircle brain sections using a hydrophobic pen previous incubation to prevent buffer leakage. Ensure that all sections are well covered with buffer.

Antibody incubation 1h 10m

3 When permeabilization is achieved, decant the buffer and incubate the sections in **primary antibody buffer** containing **diluted primary antibodies** **Overnight** at **4 °C** .

4 5m

When incubation time is finished, wash the sections 3 times with **PBS**, **00:05:00** each.

5 Incubate the cells in **secondary antibody buffer** containing **diluted secondary antibodies** **01:00:00** at **Room temperature** 1h

6 5m

Subsequently, wash the cells 3 times with **PBS**, **00:05:00** each.

7 To mount the sections, Remove as much of the remaining buffer as possible from the crystals, and with a pipette tip drop 1-2 small drops of Fluoromount-G **Electron Microscopy Sciences Catalog #17984-25** .
Subsequently, cover the sections with appropriate glass coverslips.

8 Let section air dry in the dark before imaging.