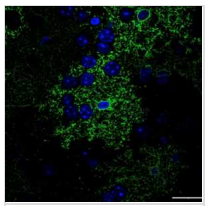

Oct 20, 2022

Immunofluorescent Labelling of Post-Mortem Rodent Brain Tissue

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dx.doi.org/10.17504/protocols.io.j8nlkkq31l5r/v1

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DISCLAIMER

Special thanks to Elize Haasdijk and Erika Sabel-Goedknegt for help with very early versions of this protocol

ABSTRACT

This protocol is to label one or several targets using a two-step immunofluorescent approach in PFA perfusion-fixed, 50-µm thick, post-mortem rodent brain tissue.

Steps 24-26 of this protocol (DAPI labelling) are optional. DAPI is a stain to label nuclei of all cells (neurons, glia) in the tissue. **Strongly recommended for independent identification of individual cells and for topographical orientation.**

DOI

dx.doi.org/10.17504/protocols.io.j8nlkkq31l5r/v1

PROTOCOL CITATION

Jeffrey Stedehouder, Shinil Raina, Stephanie J Cragg 2022. Immunofluorescent Labelling of Post-Mortem Rodent Brain Tissue. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlkkq31l5r/v1

FUNDERS ACKNOWLEDGEMENT

Medical Research Council (MRC)
Grant ID: MR/V013599/1

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

https://pubmed.ncbi.nlm.nih.gov/28922832/

KEYWORDS

Histology, immunofluorescence, postmortem, immunohistochemistry

CREATED

Jul 19, 2022

LAST MODIFIED

Oct 20, 2022

PROTOCOL INTEGER ID

67073

GUIDELINES

Every antibody and target has its own optimal binding conditions. Several portions of this protocol could be optimized for specific labelling targets.

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MATERIALS TEXT

Equipment:

- MS 3.1 digital shaker (IKA, SKU #0003319000)
- Gilson pipette (SKU #FA10005M; SKU #FA10003M; SKU #FA10006M)
- Pastuer pipette (VWR, SKU #612-1681)
- Corning® Costar® TC-Treated Multiple Well Plates (SKU #CLS3513-50EA)
- Eppendorf™ Polypropylene Graduated Microtubes (SKU #10509691)
- Wet Set Quick Dry Topcoat
- Adhesion slides, Polysine® (VWR, SKU #631-0107)

Reagents:

- Phosophate buffered saline (Sigma-Aldrich, SKU #P4417)
- Phosphate buffer: Sodium phosphate dibasic dihydrate (0.2M) (Sigma-Aldrich, SKU #71643, CAS #10028-24-7) & Sodium phosphate monobasic monohydrate (0.2M) (Sigma-Aldrich, SKU #S9638, CAS #10049-21-5)
- Normal Bovine Serum (Abcam, SKU #ab7479)
- Triton-X 100 (Sigma-Aldrich, X100, CAS #9036-19-5)
- DAPI readymade solution (Sigma-Aldrich, SKU #MBD0015, CAS #28718-90-3)
- Vectashield(Vector Labs, SKU #H-1900-10)
- Guinea pig anti-s100β primary antibody (Synaptic systems, SKU #287004, RRID #AB_2620025)
- Goat anti-ChAT primary antibody (Millipore, SKU #AB114P, RRID #AB_2313845)
- Alexa Fluor® 488 AffiniPure Donkey Anti-Guinea Pig IgG (H+L) (Jackson ImmunoResearch, SKU #706-545-148, RRID #AB_2340472)
- Donkey Anti-Goat IgG H&L (DyLight® 594) (Abcam, SKU #ab96937, RRID #AB_10680873)

DISCLAIMER:

Special thanks to Elize Haasdijk and Erika Sabel-Goedknegt for help with very early versions of this protocol

Washes 45m

1 Place 50 µm thick brain slices in a jar (or well). The jar or well should be able to hold ~2-5 ml Phosphate Buffered Saline (PBS) for proper washing.

The volume of solutions specified in this protocol are recommended for a maximum of 12 slices. If using more slices than 12, it is suggested to increase the preincubation volume to 2 ml and the incubation for primary and secondary antibodies to 1.5 ml.

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2 Add a volume (~2 ml) of PBS in each jar (or well).

Unless specified, all these steps should be done at room temperature (20-22 °C)

3 Place the jar or well on an orbital shaker for ~10-15 mins.

Shaker speed should be sufficient for the slices to move but not damage from the shaking. **Be careful not to damage the slices or leave them dry without any buffer for too long.**

4 After ~10-15 mins, remove the majority of PBS and add fresh PBS.

5 Repeat wash (steps 3 and 4) two more times for 10-15 mins each.

Preincubation 1h

6 Prepare preincubation solution containing PBS with 10% Normal Bovine Serum (NBS) and 0.5% Triton-X (total 1.5 ml per well/jar) during the last wash.

7 Remove the PBS from the last wash and add the preincubation solution.

Make sure all slices are in the solution and freely moving (e.g. not stuck to side of well/jar).

8 Leave to preincubate at room temperature for 1 h on an orbital shaker.

Primary Antibody 2d

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9 Prepare 2% Normal Bovine Serum (NBS) and 0.4% Triton-X (total 1 ml per well/jar) during the preincubation period.

10 Add the Primary Antibody to the above mix in the desired concentration. For instance, the S100β and ChAT antibodies were used at 1:1000 and 1:100, respectively.

11 Once done, remove all the preincubation solution and add the Primary Antibody Solution.

Make sure all slices are in the solution and freely moving (e.g. not stuck to side of well/jar).

12 Leave to incubate for ~48-72 h at 4º C on an orbital shaker.

Washes 45m

13 Remove all the Primary Antibody Solution and add fresh PBS.

14 Place the jar or well on an orbital shaker for ~10-15 mins.

Shaker speed should be sufficient for the slices to move but not damage from the shaking. **Be careful not to damage the slices or leave them dry without any buffer for too long.**

15 After ~10-15 mins. remove the majority of PBS and add fresh PBS.

16 Repeat wash (steps 14 and 15) two more times for 10-15 mins each.

Secondary Antibody 2h

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17 Prepare PBS with 2% Normal Bovine Serum (NBS) and 0.4% Triton-X (total 1 ml per well/jar) during the last wash.

18 Add the Secondary Antibody to the above mix in the desired concentration (commonly 1:300). For instance, Donkey anti-Guinea pig Alexa Fluor 488 and Donkey anti-Goat Dylight 594 were used at 1:300.

19 Once done, remove all the PBS from the last wash and add the Secondary Antibody Solution.

> **Make sure all slices are in the solution and freely moving (e.g. not stuck to side of well/jar).**

20 Leave to incubate for ~2-5 h at room temperature on an orbital shaker.

Washes 20m

21 Remove all the Secondary Antibody Solution and add fresh Phosphate buffer (PB).

22 Place the jar or well on an orbital shaker for ~10 mins.

> Shaker speed should be sufficient for the slices to move but not damage from the shaking. **Be careful not to damage the slices or leave them dry without any buffer for too long.**

23 After ~10 minutes, remove the majority of PB and add fresh PB.

24 Repeat wash (steps 22 and 23) one more time for ~10 mins.

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DAPI 15m

25 ⊛

Prepare DAPI solution by mixing 1 µl DAPI Readymade solution in 1 ml PB (1:1000).

26 Remove the PB from the last wash and add the DAPI Solution.

27 Leave on the orbital shaker for ~15 mins.

Shaker speed should be sufficient for the slices to move but not damage from the shaking. **Be careful not to damage the slices or leave them dry without any buffer for too long.**

Washes 20m

28 Remove all the DAPI Solution and add fresh PB.

29 Place the jar or well on an orbital shaker for ~10 mins.

Shaker speed should be sufficient for the slices to move but not damage from the shaking. **Be careful not to damage the slices or leave them dry without any buffer for too long.**

30 After ~10 minutes, remove the majority of PB and add fresh PB.

31 Repeat wash (steps 29 and 30) one more time for ~10 mins.

Mounting

32 Mount the individual slices on a glass microscope slide.

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33 Apply a small drop of Vectashield on the glass coverslip, and carefully place the coverslip on the glass slides containing the brain slices.

34 Add nail polish to the edges to seal the Vectashield shut and leave to dry before long-term storage in the fridge.

Proceed with imaging within days after completing the protocol. Under standard conditions - assuming the protocol was properly executed - the samples should remain in imaging conditions for months/up to a year, although the signal will fade over time.

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