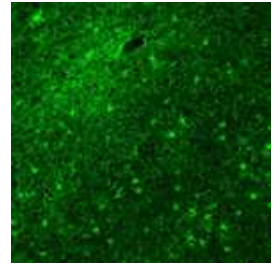


Dec 07, 2023

Immunofluorescence Staining

DOI

<https://dx.doi.org/10.17504/protocols.io.j8nlkowddv5r/v1>



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DOI: <https://dx.doi.org/10.17504/protocols.io.j8nlkowddv5r/v1>

Protocol Citation: Toby Curless, Hemanth Ramesh Nelvagal, Zane Jaunmuktane 2023. Immunofluorescence Staining. protocols.io <https://dx.doi.org/10.17504/protocols.io.j8nlkowddv5r/v1>

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Protocol status: Working

We use this protocol and it's working

Created: August 07, 2023

Last Modified: May 31, 2024

Protocol Integer ID: 86049

Keywords: ASAPCRN, immunofluorescence, brain section, brain

Funders Acknowledgements:

The Michael J. Fox Foundation for Parkinson's Research (MJFF) and the Aligning Science Across Parkinson's (ASAP) Initiative
Grant ID: ASAP-000478

Abstract

The protocol describes immunofluorescence staining on brain sections.

Materials

- 10% normal goat serum in PBS
- 100% Ethanol
- 70% Ethanol
- Antifadamountant (P36980, Thermo Fisher Scientific)
- Coverslips
- DEPC H2O
- Epifluorescence microscope (Leica) Alexa 488 filters
- PBS
- Primary and secondary antibodies
- Steamer
- Xylene

Safety warnings

- ❗ Breathing in xylene vapours in the air can cause irritation to the eyes, nose and throat and it can cause irritation, redness and swelling to the skin or eyes; and headaches, dizziness, vertigo and drowsiness. Please work to your institution approved health & safety policies, risk assessments and local procedures for the safe use, storage and disposal of xylene. Refer to the data safety sheet.

Preparation

1 Generate tissue sections using standard microtome sectioning protocols.

2 Heat tissue dry tissue sections for  01:00:00 at  60 °C

1h

De-paraffinisation and Immunofluorescence

6h 6m 30s

3

20m

Safety information

Please refer to the Xylene Data Safety Sheet and follow local approved risk assessments and protocols for safe handling and disposal of xylene.

Hazard pictograms



De-paraffinise sections by  00:05:00 min washes in xylene (x3),  00:05:00 100 % ethanol (x1),  00:05:00 70 % ethanol (x1) and  00:05:00 DEPC H₂O (x1).

4 Rinse sections in PBS.



- 5 Encircle tissue with a hydrophobic ImmEdge™ PAP pen (Vector laboratories) to contain solution.
- 6 Incubate tissue with H₂O₂ for  00:15:00 mins at room temperature. 15m
- 7 Wash tissue 3x in PBS.
- 8 Antigen retrieval – heat 1 container (250 ml) DEPC H₂O, and 1 container (250 ml) CC1 buffer to  99 °C using a steamer (BRAUN tribute collection).
- 9 Add slides to heated DEPC H₂O for 10 s, then transfer to CC1 buffer and incubate at  99 °C for  00:15:00 . 15m
- 10 Remove slides and was 3x with PBS for  00:05:00 mins total. 5m
- 11 Block sections with 10 % normal goat serum in PBS for  02:00:00 . 2h
- 12 Wash tissue in PBS.
- 13 Incubate sections in primary antibodies (antibodies diluted in PBS) at  4 °C  Overnight . 2h
- 14 Following overnight incubation, wash the sections in PBS 3x for a total of  00:15:00 and shake off excess PBS after each  00:05:00 interval. 20m
- 15 Incubate sections with 1:100 secondary antibodies (e.g., Alexa Fluor 488-conjugated anti-mouse secondary antibody (Invitrogen) and Alexa Fluor 568-conjugated anti-rabbit 30m



secondary antibody (Invitrogen)) (for ⌚ 00:30:00 mins at room temperature **in the dark**.

- 16 Wash sections in PBS 3x for a total of ⌚ 00:15:00 – whilst incubating, remove Antifade mountant (P36980, Thermo Fisher Scientific) from refrigerator and bring to room temperature. 15m
- 17 Counterstain sections with Hoechst (1:15000 (diluted in DEPC H₂O)) for ⌚ 00:00:30 sec 30s
- 18 🌡 4 °C Rinse sections in PBS 2x for a total of ⌚ 00:06:00 6m
- 19 Coverslip slides using Antifade mountant (P36980, Thermo Fisher Scientific) and store slides in the dark at 🌡 4 °C .
- 20 Capture slides on an epifluorescence microscope (Leica) using Alexa 488 filters.

