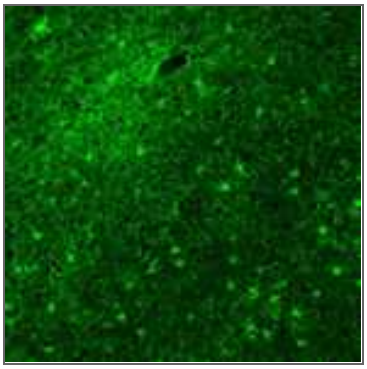

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Protocol status: Working
We use this protocol and it's working

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Immunofluorescence Staining

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ABSTRACT

The protocol describes immunofluorescence staining on brain sections.

MATERIALS

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

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Keywords: ASAPCRN

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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 or 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 assessements 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 H_2O (x1).

4 Rinse sections in PBS.

5 Encircle tissue with a hydrophobic ImmEdge^{TM} PAP pen (Vector laboratories) to contain solution.

6 Incubate tissue with $H_2O_2$for 00:15:00 mins at room temperature. 15m

7 Wash tissue 3x in PBS.

8 Antigen retrieval – heat 1 container (250 ml) DEPC H_2O, and 1 container (250 ml) CC1 buffer to 99 °C using a steamer (BRAUN tribute collection).

9 Add slides to heated DEPC H_2O for 10 s, then transfer to CC1 buffer and incubate at 99 °C for 15m 00:15:00.

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 o 20m excess PBS after each 00:05:00 interval.

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

16 Wash sections in PBS 3x for a total of 00:15:00 – whilst incubating, remove Antifade mountan 15m
(P36980, Thermo Fisher Scientific) from refrigerator and bring to room temperature.

17 Counterstain sections with Hoechst (1:15000 (diluted in DEPC H_2O)) 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.