Aug 11, 2023 Version 1

Free-floating Mouse Brain Immunohistochemistry V.1

DOI
https://dx.doi.org/10.17504/protocols.io.261ged3j7v47/v1

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Protocol Citation: Jonathan Breiter 2023. Free-floating Mouse Brain Immunohistochemistry. **protocols.io** https://dx.doi.org/10.17504/protocols.io.261ged3j7v47/v1

Protocol status: In development
We are still developing and optimizing this protocol

Created: August 09, 2023

Last Modified: June 01, 2024

Protocol Integer ID: 86178

Keywords: ASAPCRN, floating mouse brain immunohistochemistry, mouse brain immunohistochemistry this protocol, floating mouse brain, immunohistochemical staining of murine tissue, immunohistochemical staining, immunohistochemistry, murine tissue, mouse brain, superior penetration of the tissue, floating approach, tissue

Funders Acknowledgements:
ASAP
Grant ID: ASAP-000509

Abstract

This protocol enables immunohistochemical staining of murine tissue with superior penetration of the tissue by the reagents due to the free-floating approach.

Materials

10x PBS pH 7.4, Gibco, Cat. No: 70011-036
Triton X-100, Merck, Cat. No: 648466-50ML
Bovine Serum Albumin heat shock fraction, Sigma-Aldrich, Cat. No: A9647 - 100G
Normal Goat Serum, Fisher Scientific, Cat. No: 11819220
Vectashield Antifade Mounting Medium PLUS, Vector Labs, Cat. No: H-1900
Mouse mAb to Alpha-synuclein pS129 (81A), Abcam, Cat. No: ab184674, RRID:AB_2819037
Donkey anti-Mouse IgG (H+L) Alexa Fluor 568, Thermo Fisher, Cat. No: A10037, RRID: AB_2534013
Millex Filter Unit 0.22 um, Merck, Cat. No: SLGP033RS
12 Well Cell Culture Plate, Corning, Cat. No: 3513
24 Well Cell Culture Plate, Thermo Scientific, Cat. No: 144530
Netwell Permeable Supports 15mm Diameter Insert 74 um Polyester Mesh, Costar, Cat. No: 3477
Micro Slides Single Frosted 75 × 25 mm, Corning, Cat. No: 2948-75X25
Cover Glass 22 × 50 mm Thickness No. 1, VWR, Cat. No: 631-0137

Tissue Preparation

1 Remove PFA-fixed tissue from storage solution and add to mesh bottom netwell insert inside of 12 well plate that is filled with 0.22 µm filtered 1x PBS.

2 Place the 12 well plate with the tissue on a horizontal shaker and wash tissue 3× 5 min at approx. 150 rpm, moving the netwell insert to the next well down after each 5 min period to immerse the tissue in new 1x PBS. Room temperature

Buffer Preparation

3 Per well of tissue make a minimum of 3.5 mL of blocking solution, consider this is needed for blocking, primary and secondary antibody incubation. All reagents should be 0.22 µm filtered:

1x PBS
5% Normal Goat Serum
2.5% Bovine Serum Albumin
0.2% Triton-X

Make in excess and keep on ice. Keep at 4 degrees celcius overnight. 4 °C

Primary Antibody Incubation

4 In a new 12 well plate, add 2-3 mL per well of blocking solution and transfer the washed tissue sections inside their netwell inserts into these wells. Incubate for 1h - 2.5 h on horizontal shaker at approx 150 rpm. Room temperature

5 Meanwhile, dilute primary antibodies in blocking solution to appropriate concentrations and keep On ice .

E.g.
- Mouse monoclonal anti pS129 (81A) (ab184674) @ 1:750

6 Add approx 250 ul of primary antibody solution to the approriate number of wells of a 24 well plate for the number of brain sections.

7 When blocking is finished, move brain tissue sections from the netwell inserts to their appropriate primary antibody well using a fine paintbrush, being careful not to destroy the tisssue.

8 Incubate on horizontal shaker at approx. 150 rpm overnight. 4 °C

Secondary Antibody Incubation

9 Prepare a new 12 well plate with clean netwell inserts and fill all wells with 0.22 µm filtered 1x PBS.

10 Transfer brain tissue sections from 24 well plate into netwell inserts inside 12 well plate and wash 4× 10 minutes on a horizontal shaker at approx. 150 rpm.
Room temperature

11 Meanwhile, make appropriate dilutions of secondary antibody in blocking solution. Keep away from light and keep On ice .

E.g.
- Donkey anti-Mouse Alexa Fluor 568 (A10037) @ 1:500

12 Add approx 250 ul of secondary antibody solution to the approriate number of wells of a 24 well plate for the number of brain sections.

13 When washing step is finished, move brain tissue sections from the netwell inserts to their appropriate secondary antibody well using a fine paintbrush, being careful not to destroy the tisssue.

Keep the well plates covered from now to avoid bleaching of fluorophores.

14 Incubate 24 well plate on a horizontal shaker at room temperature at approx. 150 rpm for 1 h - 2.5 h. Room temperature

15 Transfer brain tissue sections from 24 well plate into netwell inserts inside 12 well plate and wash 4× 10 minutes on a horizontal shaker at room temperature at approx. 150 rpm.
Room temperature

Microscope slide preparation & Imaging

16 Plasma-clean microscope cover slips in Argon plasma for 15 minutes.

17 Using a fine paintbrush, transfer brain tissue sections from 1x PBS onto a microscope slide, using excess 1x PBS to mount multiple sections next to each other and making sure they are not folded over.

18 Leave sections on microscope slides to dry out in the dark (they will turn white).

19 Add approx. 150 uL of mounting media on top of each tissue section and apply the plasma-cleaned cover slip on top of the tissue, closing the slide.

20 Leave the mounting media to dry in the dark.

21 Seal the edges of the coverslip with nail varnish and let dry for approx. 30 minutes in the dark.

22 Immediately take finished microscope slides to imaging, avoiding unecessary light exposure.