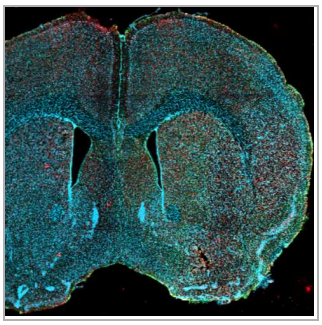

NOV 23, 2022

WORKS FOR ME 1

Staining of Gfap, Iba1,and NeuN on PFA-fixed mouse brain sections

COMMENTS 0

DOI
dx.doi.org/10.17504/protocols.io.4r3l27q5pg1y/v1

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ABSTRACT

Staining protocol for Gfap, Iba1, and NeuN on PFA-fixed mouse brain sections.

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PROTOCOL CITATION

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GUIDELINES

Read the full protocol before starting the procedure.

Note that this protocol uses 3 hours (room temperature) incubation for primary antibodies.

Works with the listed antibodies and dilutions. For other references, preliminary tests are highly recommended.

MATERIALS TEXT

Equipment

ImmEdge® Hydrophobic Barrier PAP Pen	NAME
Vector	BRAND
H-4000	SKU
https://vectorlabs.com/products/histology/immedge-hydrophobic-barrier-pen	LINK
Immunohistochemistry / Immunocytochemistry, Immunofluorescence, In situ hybridization	SPECIFICATIONS

Permeabilization solution = [M] 0.3 % (v/v) Triton Triton X-100 **Sigma Aldrich Catalog #T8787-50ML** / = [M] 0.1 % (v/v) TWEEN 20 **Sigma Aldrich Catalog #P7949** In 3 steps / [M] 0.3 Molarity (m) Glycine **Contributed by users** in **1x PBS**

Antibody buffer = [M] 0.1 % (v/v) TWEEN 20 **Sigma Aldrich Catalog #P7949** In 3 steps / [M] 1 % (v/v) Goat serum **Sigma Aldrich Catalog #G9023** Step 4 [M] 1 Mass / % volume Bovine Serum Albumin (BSA) **Sigma Aldrich Catalog #A7906** Step 4

Primary antibodies:

GFAP Monoclonal Antibody (2.2B10) **Thermo Fisher Scientific Catalog #13-0300**
Anti Iba1 Rabbit antibody **FUJIFILM Wako Pure Chemical Corporation Catalog #019-19741**
Anti-NeuN Antibody **Emd Millipore Catalog #ABN91**

Secondary antibodies:

Goat anti-Rat IgG (H L) Cross-Adsorbed Secondary Antibody Alexa Fluor 488 **Thermo Fisher Scientific Catalog #A-11006**
Cy3-AffiniPure Goat Anti-Rabbit IgG (H L) antibody **Jackson Immunoresearch Catalog #111-165-003**
Goat anti-Chicken IgY (H L) Cross-Adsorbed Secondary Antibody Alexa Fluor Plus 647 **Thermo Fisher Scientific Catalog #A32933**
DAPI (46-Diamidino-2-Phenylindole Dilactate) **Invitrogen - Thermo Fisher Catalog #D3571**

Mounting media:

Fluoromount-G **Electron Microscopy Sciences Catalog #17984-25** Step 9

SAFETY WARNINGS

DAPI is highly toxic. Handle it with care.

20m

Tissue preparation and blocking

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20m

1 Take out sections from -80 and place them in an incubator/plate for 00:20:00 at 37 °C. This step is performed to ensure tissue attachment to the crystal slides.

10m

2 2. Draw a hydrophobic barrier on each slide using **ImmEdge®Hydrophobic Barrier PAP Pen** and let dry for about 00:10:00 minutes. This will prevent buffers from escaping the tissue area.

Note

There are other hydrophobic pens on the market. However, this one is recommended for its quality, consistency, and durability.

30m

3 To initially rehydrate and permeabilize the tissue, place the slides in a slide jar containing the **permeabilization solution** with shaking for 00:30:00

Note

The proposed permeabilization solution contains **glycine**, suitable to reduce PFA-related autofluorescence, especially in the 488 channel.

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1h

4 To prevent unspecific binding, incubate brain sections in **permeabilization solution** containing [M] 5 % (v/v) Goat serum **Sigma Aldrich Catalog #G9023** and [M] 1 Mass / % volume Bovine Serum Albumin (BSA) **Sigma Aldrich Catalog #A7906** for 01:00:00 at Room temperature.

Note

Please note that blocking serum must be chosen according to **secondary antibody species**. For the procedure depicted in this protocol, all secondary antibodies are raised in goat. However, donkey secondary antibodies will be also suitable.

Additionally, consider as well **avoiding BSA** when **primary antibodies** come from goat or sheep. BSA can generate unspecific background.

3h

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Antibody incubation

3h

5 When blocking is finished, decant the buffer (no washing is required) and incubate **primary antibodies** in **antibody buffer** for 03:00:00 at Room temperature according to **table 1**.

A	B	C	D	E
Antibody	Company	Reference	Specie	Dilution
Gfap	Invitrogen	13-0300	Rat	1:500
Iba1	Wako	019-19741	Rabbit	1:500
NeuN	Millipore	ABN91	Chicken	1:300

Table 1. Primary antibodies

Note

Please note that the antibody buffers **do not contain Triton X-100**. The reason is that this detergent tends to break the hydrophobic barrier, and the sections may not be adequately incubated. In addition, because sufficient permeabilization has already been performed previously, this detergent is not contemplated in this step.

Please note that the proposed antibody incubation lasts only **3 hours**. Although in most cases, staining protocols recommend overnight incubation for primary antibodies, the tests performed in our lab disclosed that, under the reported conditions, labeling for NeuN and Iba1 is superior at room temperature.

Also, the cited **Iba1 antibody** shows evident labeling and specificity superiority compared to others available in the market; therefore, highly recommended.

5m

6 When primary antibody incubation is finished, wash the sections 00:05:00 5x with [M] 0.05 % (v/v) TWEEN 20 **Sigma Aldrich Catalog #P7949** in **PBS.**

1h

7 Incubate **secondary antibodies** in [M] 0.1 % (v/v) TWEEN 20 **Sigma Aldrich Catalog #P7949** for 01:00:00 at Room temperature according to **table 2.**

A	B	C	D	E
Antibody	Company	Reference	Channel	Dilution
Goat anti-rat	Invitrogen	A11006	488	1:500

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A	B	C	D	E
Goat anti-rabbi	Jackson	111-165-003	Cy3	1:500
Goat anti-chick	Invitrogen	A32933	647	1:500
Dapi	Invitrogen	D3571	405 (Dapi)	1:5000

Table 2. Secondary antibodies

10m

8 When secondary antibody incubation is finished, wash the sections 00:05:00 3x with [M] 0.05 % (v/v) TWEEN 20 **Sigma Aldrich Catalog #P7949** in **PBS.** Follow this with 00:05:00 2x washes with **PBS** to remove all detergent traces.

9 Clean the remaining buffer on the slides using absorbent tissue and mount the sections with a drop flo Fluoromount-G **Electron Microscopy Sciences Catalog #17984-25** .

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