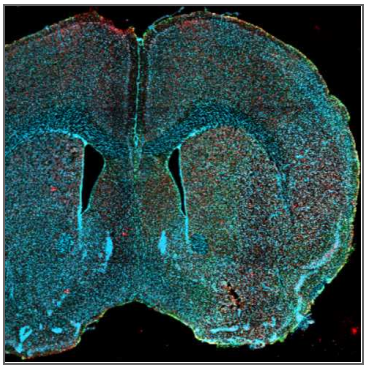

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Protocol status: Working
We use this protocol and it's working

Created: Sep 01, 2023

Staining of GFAP and IBA1 in PDGFR-B/Td-tomato brain sections

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Protocols

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ABSTRACT

This protocol is suitable for the staining of GFAP and IBA1 in PDGFR-B/Td-Tomato fixed mouse brain sections.

GUIDELINES

Read the entire protocol before starting the procedure.

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

Do not submit the tissue to antigen retrieval or other acidic solutions to preserve theTd-tomato signal.

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

MATERIALS

Last Modified: Sep 01, 2023

PROTOCOL integer ID: 87251

Keywords: Staining, GFAP, IBA1, Immunofluorescence

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** /

[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**

/ [M] 1 % (v/v)

Normal Donkey Serum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D9663-10ml**

[M] 1 Mass / % volume

Bovine Serum Albumin (BSA) **Sigma Aldrich Catalog #A7906**

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**

Secondary antibodies:

Alexa Fluor® 647 AffiniPure Donkey Anti-Rat IgG (H L) **Jackson ImmunoResearc Laboratories, Inc. Catalog #712-605-153**

Anti-Rabbit **Invitrogen - Thermo Fisher Catalog #A21206**

DAPI (46-Diamidino-2-Phenylindole Dilactate) **Invitrogen - Thermo Fisher Catalog #D3571**

Mounting media:

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

PROTOCOL MATERIALS

Alexa Fluor® 647 AffiniPure Donkey Anti-Rat IgG (H L) **Jackson ImmunoResearc Laboratories, Inc. Catalog #712-605-153**

Materials

Glycine **Contributed by users** Materials

TWEEN 20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P7949**

In Materials, Materials and 3 steps

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

Materials, Step 9

Anti-Rabbit **Invitrogen - Thermo Fisher Catalog #A21206** Materials

Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T8787-50ML**

Materials

Bovine Serum Albumin (BSA) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7906**

Materials

Normal Donkey Serum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D9663-10ml**

Materials

SAFETY WARNINGS

DAPI is highly toxic. Handle it with care.

Tissue preparation and blocking

20m

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

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. 10m

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

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 30m

> Note
>
> The proposed permeabilization solution contains **glycine**, which is suitable for reducing PFA-related autofluorescence, especially in the 488 channel.
>
> Antigen retrieval or any treatment with acid is not recommended given that it destroys the endogenous Td-tomato signal from PDGFR-B+ cells.

.

4 To prevent unspecific binding, incubate brain sections in **permeabilization solution** containing 1h
[M] 5 % (v/v)
Normal Donkey Serum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D9663-10ml** 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 donkey. However, goat secondary antibodies will also be suitable.

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

Antibody incubation

3h

5

3h

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

Table 1. Primary antibodies

Note

Please note that antibody buffers **do not contain Triton X-100**. This detergent tends to break the hydrophobic barrier and the sections may not be adequately incubated. In addition, given that the sections are already permeabilized, we do not contemplate the use of this detergent.

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 showed better efficiency at room temperature.

The indicated **Iba1 antibody** is superior to others available in the market.

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.** 5m

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.** 1h

	A	B	C	D	E
	Antibody	Company	Reference	Channel	Dilution
	Donkey anti-rat	Jackson	712-605-153	647	1:500
	Donkey anti-rabbit	Invitrogen	A21206	488	1:500
	Dapi	Invitrogen	D3571	405 (Dapi)	1:5000

Table 2. Secondary antibodies

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. 10m

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.