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

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

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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

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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T8787-50ML / = [M] 0.1 % (v/v)
TWEEN 20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P7949 /
[M] 0.3 Molarity (M) Glycine in **1x PBS**

Antibody buffer = [M] 0.1 % (v/v) TWEEN 20 **Merck MilliporeSigma (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) **Merck MilliporeSigma (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 ImmunoResearch 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

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

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

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

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

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

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

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

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

Anti Iba1 Rabbit antibody **FUJIFILM Wako Pure Chemical Corporation Catalog #**019-19741

GFAP Monoclonal Antibody (2.2B10) **Thermo Fisher Scientific Catalog #**13-0300

Glycine

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. This step is performed to ensure tissue attachment to the crystal slides. 20m

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.

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4 To prevent unspecific binding, incubate brain sections in **permeabilization solution** containing [M] 5 % (v/v) 1h

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

and [M] 1 Mass / % volume

Bovine Serum Albumin (BSA) **Merck MilliporeSigma (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 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**.

3h

	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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P7949** in **PBS.** 5m

7 Incubate **secondary antibodies** in [M] 0.1 % (v/v) TWEEN 20 **Merck MilliporeSigma (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 **Merck MilliporeSigma (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** .