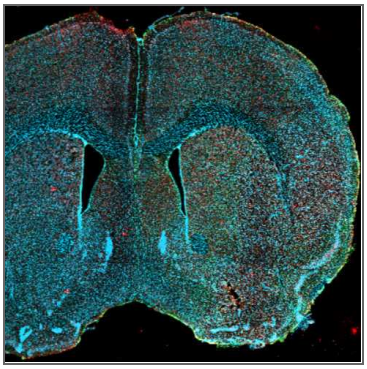

SEP 05, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.x54v9p2b1g3e/v1

Protocol Citation: Daniel Manrique-Castano 2023. Staining of CD31 and CD13 in PDGFR-B/Td-tomato brain sections. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v9p2b1g3e/v1

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

Created: Sep 05, 2023

Staining of CD31 and CD13 in PDGFR-B/Td-tomato brain sections

Daniel Manrique-Castano[1]

[1]Université Laval

Daniel Manrique-Castano
Université Laval

ABSTRACT

This protocol is suitable for the staining of CD31 and CD13 in PDGFR-B/Td-Tomato fixed mouse brain sections.

GUIDELINES

Read the entire protocol before starting the procedure.

Note that this protocol uses free-floating sections to enhance CD31 staining.

This protocol uses sequential staining to avoid cross-reactivity between CD31 and CD13.

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 05, 2023

PROTOCOL integer ID: 87394

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:

Mouse Aminopeptidase N/CD13 Antibody **R&D Systems Catalog #AF2335**

Purified Rat Anti-Mouse CD31 **BD Biosciences Catalog #550274**

Secondary antibodies:

Donkey anti-Goat IgG (H+L) **Thermo Fisher Scientific Catalog #A-11055**

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

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

Mounting media:

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

PROTOCOL MATERIALS

Donkey anti-Goat IgG (H L) **Thermo Fisher Scientific Catalog #A-11055**

Materials

Purified Rat Anti-Mouse CD31 **BD Biosciences Catalog #550274** Materials

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

Materials

Mouse Aminopeptidase N/CD13 Antibody **R&D Systems Catalog #AF2335**

Materials

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

Materials, Step 12

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

Materials

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

In Materials, Materials and 7 steps

Glycine **Contributed by users** Materials

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

Materials

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

Materials

SAFETY WARNINGS

DAPI is highly toxic. Handle it with care.

Tissue preparation and blocking

20m

1 Extract the sections from the anti-freeze media and rinse them in PBS using a 24-well plate.

2 Aspirate the PBS and incubate the sections in the **Blocking solution** for 01:00:00 at Room temperature . 1h

Antibody incubation 12h

3 When blocking is finished, aspirate the buffer (no washing is required) and incubate CD31 (BD Bioscience, 550274, 1:200) in **antibody buffer** for Overnight at 4 °C. 3h

Note

Since the sections are already permeabilized, please note that antibody buffers do not contain Triton X-100

Please note that the proposed antibody incubation includes only **CD31. CD13** will be incubated after.

4 When primary antibody incubation is finished, aspirate the media and wash the sections 5m

00:05:00 5x with [M] 0.05 % (v/v) TWEEN 20 Sigma Aldrich Catalog #P7949 in **PBS.**

5 Incubate **Donkey α Goat 488 (Invitrogen, A-11055, 1:500) secondary antibody** in [M] 0.1 % (v/v) 1h

TWEEN 20 Sigma Aldrich Catalog #P7949 for 01:00:00 at Room temperature

6 When secondary antibody incubation is finished, aspirate the media and wash the sections 5m

00:05:00 5x with [M] 0.05 % (v/v) TWEEN 20 Sigma Aldrich Catalog #P7949 in **PBS.**

Tissue blocking

7 Perform a second blocking step using **5% Donkey serum** in [M] 0.1 % (v/v) TWEEN 20 Sigma Aldrich Catalog #P7949 in **PBS.**

Antibody incubation

12h

8 When blocking is finished, aspirate the buffer (no washing is required) and incubate CD13 (R&D, AF2335, 1:300) in **antibody buffer** for 03:00:00 at Room temperature. 3h

Note

Please note that this antibody incubation is performed differently from the first one (3 hours at room temperature).

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

10 Incubate **Donkey α Rat 647 (Jackson, 712-605-153, 1:300) secondary antibody** in [M] 0.1 % (v/v) TWEEN 20 Sigma Aldrich Catalog #P7949 for 01:00:00 at Room temperature. Add DAPI **(Invitrogen, D3571, 1:5000)** 1h

Wash and mounting

12h

11 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

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