

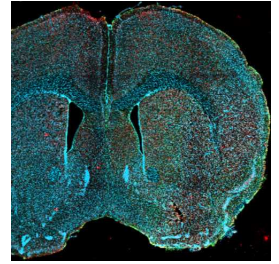


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🌐 Staining of CD31 and CD13 in PDGFR-B/Td-tomato brain sections

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Protocol status: Working

We use this protocol and it's working

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Keywords: Staining, GFAP, IBA1, Immunofluorescence, cd13 in pdgfr, tomato brain section, tomato brain sections this protocol, cd31, mouse brain section, cd13, fixed mouse brain section, pdgfr, staining, suitable for the staining

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 the Td-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:

☒ 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 ImmunoResearch 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

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

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

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

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⊗ Glycine

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

Troubleshooting



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 .

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 .



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 00:05:00 5x with 0.05 % (v/v) TWEEN 20 Merck MilliporeSigma (Sigma-Aldrich) Catalog #P7949 in **PBS**.
- 5 Incubate **Donkey α Goat 488 (Invitrogen, A-11055, 1:500) secondary antibody** in 0.1 % (v/v) TWEEN 20 Merck MilliporeSigma (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 00:05:00 5x with 0.05 % (v/v) TWEEN 20 Merck MilliporeSigma (Sigma-Aldrich) Catalog #P7949 in **PBS**.

5m



1h



5m



Tissue blocking

- 7 Perform a second blocking step using **5% Donkey serum** in [IM] 0.1 % (v/v) TWEEN 20 Merck MilliporeSigma (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 [IM] 0.05 % (v/v) TWEEN 20 Merck MilliporeSigma (Sigma-Aldrich) Catalog #P7949 in **PBS**.
- 10 Incubate **Donkey α Rat 647 (Jackson, 712-605-153, 1:300) secondary antibody** in [IM] 0.1 % (v/v) TWEEN 20 Merck MilliporeSigma (Sigma-Aldrich) Catalog #P7949 for 01:00:00 at Room temperature . Add DAPI (**Invitrogen, D3571, 1:5000**)

5m

1h

Wash and mounting

12h

- 11 When secondary antibody incubation is finished, wash the sections 00:05:00 3x with [IM] 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.
- 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 .

10m