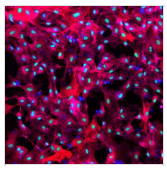

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Human Brain Vascular Pericytes (HBVP) Fixation and Staining

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

This protocol is suitable for the fixation and staining of cultured Human Brain Vascular Pericytes (HBVP).

PROTOCOL CITATION

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KEYWORDS

cell culture, Human Brain Vascular Pericytes (HBVP), cell fixation, cell staining, cell immunohistochemistry , neuroscience

IMAGE ATTRIBUTION

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GUIDELINES

Please read the whole protocol before starting the procedure.

MATERIALS TEXT

2% Paraformaldehyde (PFA)

Preparation:

1. For **1 L** of **2% Paraformaldehyde** (PFA), place **800 mL** of 1X PBS (Phosphate-buffered saline) **Contributed by users** in a glass beaker on a stir plate in a ventilated hood. Heat while stirring to approximately 60 °C. **Avoid boiling**.
2. Add **20 g** of paraformaldehyde powder to the heated solution.
3. The powder will not immediately dissolve into the solution. Slowly raise the pH by adding **[M]1 Molarity (M) NaOH** dropwise from a pipette until the solution clears.
4. Once the paraformaldehyde is dissolved, the solution should be **cooled and filtered**.

5. Adjust the volume 1 L with 1X PBS (Phosphate-buffered saline) **Contributed by users**
6. Recheck the pH, and adjust it with small amounts of **HCl (1ML)** to pH **7.2**
7. The solution can be aliquoted and frozen or stored at 4 °C for up to one month.

Dulbecco's phosphate-buffered saline (DPBS)

Dulbeccos phosphate-buffered saline (DPBS) **Gibco - Thermo Fischer Catalog #14190144**

Blocking-Permeabilization buffer

1X PBS (Phosphate-buffered saline) **Contributed by users**

[M] **0.1 % (v/v)** Triton-X100 **Contributed by users**

Tween 20 **Bio-rad**

[M] **0.05 % (v/v)** **Laboratories Catalog #170-6606-MSDS**

Glycine **Sigma –**

[M] **0.3 Molarity (M)** **Aldrich Catalog #G8898**

Primary antibody buffer

1X PBS (Phosphate-buffered saline) **Contributed by users**

Tween 20 **Bio-rad**

[M] **0.1 % (v/v)** **Laboratories Catalog #170-6606-MSDS**

Normal Goat Serum **Gibco - Thermo**

[M] **5 % (v/v)** **Fischer Catalog #LSPCN5000** or

Donkey Serum **Emd**

Millipore Catalog #S30-100ML depending on your secondary antibody species.

Secondary antibody buffer

1X PBS (Phosphate-buffered saline) **Contributed by users**

Normal Goat Serum **Gibco - Thermo**

[M] **1 % (v/v)** **Fischer Catalog #LSPCN5000** or

Donkey Serum **Emd**

Millipore Catalog #S30-100ML

SAFETY WARNINGS

Paraformaldehyde (PFA) is a highly toxic subtance. Manipulation should be performed carefully and according to security measurements.

DISCLAIMER:

> This protocol was adapted by Daniel Manrique-Castano, based on experimental procedures performed at the Laboratory of Neurovascular Interactions at Université Laval (https://elalilab.com/)

BEFORE STARTING

It is recommended that all reagents are warmed at **37 °C** prior contact with cells. Exposure of cultured pericytes to cold reagents may result in cell damage or detachment from the plate.

Cell fixation 37m

1 30m

When cultured pericytes have reached the desired confluence on **Poly-D-Lysine** or **Matrigel-coated** glass coverslips, they can be fixed and prepared for staining. Before starting cell manipulation, warm **2% Paraformaldehyde (PFA)** at **37 °C** for **00:30:00** .

This procedure is unsuitable to perform RNA or protein extraction.

2 7m

To avoid cell damage by the abrupt change between the culture media's and the fixation solution's osmolarity, add **500 µl** of **2% PFA** during **00:02:00** . After, suction the medium and incubate the cells with **500 µl** of **2% PFA** during **00:05:00** at **Room temperature** .

When adding substrates, pipette on the lateral walls of each wheel and not directly on to of the cells to avoid cell detachment or damage.

3 Under the microscope, verify cell adherence to the glass coverslips

4

Aspirate **PFA**, and Rinse three times with **DPBS** to remove fixative residuals.

5 Keep cells in **1 mL** DPBS at **4 °C** until staining.

Cell staining 1h 25m

6 Recover cells from **4 °C** and aspirate the **DPBS**.

7 15m

To permeabilize and block for unspecific staining, incubate the cells in **500 µl** of **Blocking-Permeabilization buffer** (see materials) for **00:15:00** at **Room temperature**

8

Aspirate the Blocking-permeabilization solution, and incubate the cells in **primary antibody buffer** containing diluted **primary antibodies** **Overnight** at **4 °C** .

9 When incubation time is finished, wash the cells 3 times with **PBS**, **00:05:00** each. 5m

10 Suction PBS, and incubate the cells with **secondary antibody buffer** containing diluted **secondary antibodies** **01:00:00** at **Room temperature** . 1h

11 Subsequently, wash the cells 3 times with **PBS**, **00:05:00** each. 5m

12 Finally, carefully detach the glass coverslip from the wheel plate using forceps, and place the cell surface facing a glass slide containing a small drop of Fluoromount-G **Electron Microscopy Sciences Catalog #17984-25** mounting media.

13 Left the mounting dry at **Room temperature** , and store the samples at **4 °C** for further imaging.