Version 3

Feb 01, 2021

Human Brain Vascular Pericytes (HBVP) Culture and Plating V.3

Rayan Khaddaj[1], Maxime Bernad[1], Daniel Manrique-Castano[1]

[1]Université Laval

1 Works for me dx.doi.org/10.17504/protocols.io.brxjm7kn

Daniel Manrique-Castano
Université Laval

ABSTRACT

This protocol implements the culture of Human Brain Vascular Pericytes (HBVP) and is suitable for several research techniques, including immunohistochemistry, flow cytometry, and Protein or RNA analysis.

DOI

dx.doi.org/10.17504/protocols.io.brxjm7kn

PROTOCOL CITATION

Rayan Khaddaj, Maxime Bernad, Daniel Manrique-Castano 2021. Human Brain Vascular Pericytes (HBVP) Culture and Plating. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.brxjm7kn
Version created by Daniel Manrique-Castano

KEYWORDS

Pericytes, Human Brain Pericytes, Cell Culture, Neuroscience

CREATED

Jan 29, 2021

LAST MODIFIED

Feb 01, 2021

PROTOCOL INTEGER ID

46795

GUIDELINES

Please read the whole protocol before starting the procedure.

MATERIALS TEXT

Human Brain Vascular Pericytes (HBVP)
ScienCell (Cat#1200)

Cell Culture Flask
Corning® 75cm² U-Shaped Canted Neck Cell Culture Flask with Vent Cap (Cat# 430641U)

Pericyte complete medium (PCM)

For **50 mL** of **PCM**:

Dulbeccos Modified Eagle Medium (DMEM) **Wisent**

1. **48 mL** of **Bioproducts Catalog #319-005-CL**

Fetal Bovine Serum (FBS) **Wisent**

2. **1 mL** of **Bioproducts Catalog #920055**

Penicillin-Streptomycin (10000 U/mL) **Gibco - Thermo**

3. **0.5 mL** of **Fischer Catalog #15140122**

Pericyte Growth Supplement

4. **0.5 mL** of (PGS) **ScienCell Catalog #1252**

Trypsin-EDTA solution

Trypsin-EDTA

solution **Sigma Catalog #T4049** . Make small aliquots to avoid repeated thaw-freezing cycles.

Dulbecco's phosphate-buffered saline (DPBS)

Dulbeccos phosphate-buffered saline (DPBS) **Gibco - Thermo**

Fischer Catalog #14190144

Matrigel

Matrigel **Corning Catalog #356231** . Make small aliquots to avoid repeated thaw-freezing cycles.

Syringe filter
Syringe filter, Filtropur S, PES, pore size: 0.2 µm, for sterile filtration (Sarstedt, cat# 83.1826.001)

Pre-treated German Glass Coverslips
(Emsdiasum, Cat#72291-03)

SAFETY WARNINGS

All reagents and instruments employed in this protocol must be sterile, and the cell manipulation must be performed in a sterile/decontaminated cell culture hood.

DISCLAIMER:

> The whole protocol was established by Rayan Khaddaj and Maxime Bernard at the Laboratory of Neurovascular Interactions (https://elalilab.com/) at the Department of Psychiatry and Neuroscience, Université Laval (Québec, Canada).

BEFORE STARTING

It is pivotal that all the reagents used in this protocol are warmed at **37 °C** prior contact with cells. Exposure of

Citation: Rayan Khaddaj, Maxime Bernad, Daniel Manrique-Castano (02/01/2021). Human Brain Vascular Pericytes (HBVP) Culture and Plating. https://dx.doi.org/10.17504/protocols.io.brxjm7kn

cultured pericytes to cold reagents results in cell stress and death.

Cell Culture 30m

30m

1

Prepare and warm **50 mL** of **Pericyte Complete Medium** (PCM, see materials section) at **37 °C** for **00:30:00** .

2m

2

Retrieve form **-130 °C** the **Human Brain Vascular Pericytes (HBVP)** and place them at **37 °C** for **00:02:00** .

As frozen HBVP contain DMSO, cell exposure to this reagent at **37 °C** should be minimal. Therefore, incubate only as long as necessary until the aliquot is in liquid form.

3 Place **1 mL** (~500.000 cells) of HBVP in a **75cm² Cell Culture Flask** filled with **9 mL** of **PCM.**

Cell culture can also be performed in smaller Cell Culture Flasks if fast confluence or reduced cell number is required. In those cases, adjust the PCM volume (i.e **4 mL** for 25cm² Cell Culture Flask).

4 Perform gently crosswise movements to spread the cells over the container's surface, and visually **check cell dispersion** under a microscope.

5

Incubate the cells at **37 °C** until reaching a **confluence of 70-80%** before proceeding with cell plating.

In a 75cm² Cell Culture Flask it is expected that 70-80% confluence results in **6-7 million cells**.

When HBVP are taken from -130 frozen aliquots, the PCM must be replaced daily during the first two days to eliminate any traces of DMSO (see step 20). After, replace the medium every two days until the desired confluence is reached.

Citation: Rayan Khaddaj, Maxime Bernad, Daniel Manrique-Castano (02/01/2021). Human Brain Vascular Pericytes (HBVP) Culture and Plating. https://dx.doi.org/10.17504/protocols.io.brxjm7kn

Glass Coverslip Preparation

6

The steps contained in this section (Glass Coverslip Preparation) are required if the experimental approach entails **immunohistochemistry**. Otherwise, If mounting in coverslips is not necessary (i.e. protein extraction), the researcher can proceed to cell detachment.

Here, the Glass Coverslip coating was performed with Matrigel, for specific purposes. In most scenarios, **Poly-D-Lysine coated** coverslips are used. To use **Poly-D-Lysine,** prepare in a sterile environment **1 mg** / **1 mL** Poly-D-Lysine hydrobromide **Sigma –** (Mili-Q water) of **Aldrich Catalog #P6407** and filter it using a **Syringe filter.** Cover the Glass Coverslip with **100 µl** or **200 µl** of the diluted **Poly-D-Lysine** and incubate at **37 °C** for **01:00:00** . Subsequently, wash with sterile DPBS. At this point, coverslips are ready for cell plating.

7 Slowly thaw aliquoted **Matrigel** by transferring it from **-20 °C** to a recipient with minced ice.

8 Dilute 1:30 the **Matrigel** in **On ice** cooled **DPBS** according to the required volume per wheel (**200 µl** for 12 wheels plate, **400 mL** for 6 wheels plate) and filter the solution using a sterile **Syringe** and a **2 µl Syringe filter.**

9 Place cold (stored at **-20 °C**) **Glass Coverslips** in each wheel and add **1 mL 70% ethanol** for **00:05:00** with subsequent suction. 5m

10 30m

On ice , add **200 µl** for 12 wheels plate or **400 mL** for 6 wheels plate of filtered matrigel and incubate the plate at **37 °C** for **00:30:00** .

11

When matrigel incubation is finished, wash the wheels three times with PCM (**200 µl** for 12 wheels plate, **500 mL** for 6 wheels plate) and subsequently, aspirate the media.

Cell Detachment 35m

12 30m

Citation: Rayan Khaddaj, Maxime Bernad, Daniel Manrique-Castano (02/01/2021). Human Brain Vascular Pericytes (HBVP) Culture and Plating. https://dx.doi.org/10.17504/protocols.io.brxjm7kn

Before starting the procedure, make sure that **PCM**, **DPBS**, and **Trypsin-EDTA solution** are warmed at **37 °C** for **00:30:00**

13 

When cells have reached 70-80% confluence (6-7 million cells), take them from the incubator, carefully suction the **PCM**, and wash 1 time with **DPBS.**

14 

5m

After suctioning DPBS, add **3 mL** of **trypsin-EDTA solution** and incubate at **37 °C** for **00:05:00** to achieve cell detachment from the flask.

If culture is performed in a smaller Cell Culture Flask, adjust the trypsin-EDTA volume accordingly. For 25cm² Cell Culture Flask, **1.5 mL** of the solution is enough.

15 Check under the microscope that cell detachment was achieved, and add **9 mL** of **PCM** (three times the trypsin-EDTA volume) to stop the chemical reaction.

If culture is performed in a smaller Cell Culture Flask, adjust the PCM volume to be three times the trypsin-EDTA volume.

16 

Repeatedly pipette the medium contained in the Cell Culture Flask to ensure complete detachment of cells that may still be adhered to the surface.

17 Suction the whole culture media containing the detached cells, and place it in a **50 mL** sterile **Falcon tube**.

18 

To separate the cells from the PCM and remaining trypsin-EDTA solution, centrifugate at **1000 rpm, Room temperature , 00:05:00 , Acceleration/deceleration speed= 6**

19 

When centrifugation is finished and cells are attached to the bottom of the Falcon tube, carefully **remove the supernatant** and add **PCM** with gently pipetting to resuspend the cells (check cases).

Citation: Rayan Khaddaj, Maxime Bernad, Daniel Manrique-Castano (02/01/2021). Human Brain Vascular Pericytes (HBVP) Culture and Plating. https://dx.doi.org/10.17504/protocols.io.brxjm7kn

Known cell number
This protocol estimates that under a confluence of 70-80% in a 75cm2 Cell Culture Flask, the researcher can retrieve a total of 7 million cells. In this scenario, cells are resuspended in 7 ml PCM to obtain 1 million cells/ml.

Cell counting
If required or desired, the researcher can conduct at this point manual or automatic cell counting according to equipment availability. This is usually done by 1:1 incubation of 10 ul of Trypan Blue and a 1/10 diluted cell sample. Then, resuspension in PCM is performed according to the desired cell concentration per milliliter.

20 At this point, cells are ready to be plated (See next section) or frozen. If the researcher wishes to freeze the cells, they must be resuspended in **PCM without FBS** and add 20% v/v **Dimethyl Sulfoxide (DMSO)**.

20.1 To freeze the cells, add **1 mL** (1 million cells) of cell suspension in **1.5 mL** Eppendorf tubes, and 1d place them in a recipient filled with isopentane. Transfer the recipient to a **-80 °C** refrigerator for **24:00:00** , and finally place them at **-130 °C** for further use.

During the freezing and thaw cycles, a loss of ~50% of cells is expected. Therefore, it is recommended to freeze 1 million cells, in order to seed at least 500.000 cells in the next passage. (see steps 2-3).

Cell Plating

21 Add **1 mL** (for 6 wheels plate) or **750 mL** (for 12 wheel plates) of **PCM** to each wheel containing the **Matrigel Coated Glass Coverslips** (see Glass Coverslip Preparation section), followed by **0.5 mL** (for 6 wheels plate) or **250 mL** (for 12 whell plate) of **PCM-suspended pericytes**.

22 

Incubate the wheel plate at **37 °C** until the desired confluence is achieved.

70-80% Confluency in 6 or 12 wheels plates is achieved in 1-3 days depending on the plated cell density.

Citation: Rayan Khaddaj, Maxime Bernad, Daniel Manrique-Castano (02/01/2021). Human Brain Vascular Pericytes (HBVP) Culture and Plating. https://dx.doi.org/10.17504/protocols.io.brxjm7kn