BBB Stem Cell Model

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Single Cell Seeding of BBB Stem Cell Model

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1 Works for me dx.doi.org/10.17504/protocols.io.8j9hur6

Neurodegeneration Method Development Community

ABSTRACT

Standardized single cell seeding protocol for Blood-Brain Barrier (BBB) differentiation.

ATTACHMENTS

Standardized_single_cell_seeding_protocol_for_BBB_differentiation_(Lippmann_Lab_updates).pdf

MATERIALS

NAME	CATALOG #	VENDOR
UltraPure™ DNase/RNase-Free Distilled Water	10977023	Thermo Fisher Scientific
Gibco™ DPBS no calcium no magnesium	14190144	Thermo Fisher Scientific
StemPro™ Accutase™ Cell Dissociation Reagent	A1110501	Thermo Fisher Scientific
Countess™ II Automated Cell Counter	AMQAX1000	Thermo Fisher
Y-27632 dihydrochloride (Rock Inhibitor)	1254/10	R&D Systems
Countess™ Cell Counting Chamber Slides	C10312	Thermo Fisher Scientific

MATERIALS TEXT

- Corning tissue culture plates
- 15 ml conical tubes
- Microfuge tubes

- E8 media prepared in-house

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

BEFORE STARTING

- Matrigel Plates should be ready to use at start of procedure.
- Procedure was optimized using IMR90-4 pluripotent stem cells. The procedure has successfully been extended to CC3, CD12, SM14, and DSP-mEGFP pluripotent stem cells.

Citation: Ethan Lippmann, Hannah Wilson, Emma Neal (03/24/2020). Single Cell Seeding of BBB Stem Cell Model. https://dx.doi.org/10.17504/protocols.io.8j9hur6

Reagent Preparation

1

- ROCK inhibitor:

Make **10 Milimolar (mM)** working stock solution by diluting **10 mg ROCK inhibitor** into **3.12 ml ultrapure distilled water** . Use at 1:1000 for **10 Micromolar (µM)** final concentration. Aliquots can be stored long term at **-80 °C** for up to 1 year and frozen/thawed as many times as necessary.

- E8 media

Seeding cells for BBB differentiation using single cell seeding (Day -1)

2 Manually transfer spent medium to a 15 ml conical. Save **1 ml** of media for every well being passaged.

3

Wash each well once with **2 ml PBS** .

4

Add **1 ml accutase** (warmed to **Room temperature**) to each well.

5

Incubate at **37 °C** until the cells are beginning to detach (approx. **00:03:00** — **00:05:00**)

6 Using p1000, collect cells, and spray gently over surface **2 — 3x** to dislodge any remaining cells.

Pipetting more than this will reduce cell viability.

7 Collect cells in the 15 ml conical containing spent medium.

8 Spin down cells for **00:04:00** — **00:05:00** at **1000 rpm** .

9

Aspirate media, resuspend cells in **1 ml E8 medium** . Thoroughly triturate 2 — 3 times using p1000 to yield single cell suspension.

10

Take **10 µl** of cells to count, drawing from the middle of the sample to prevent bias from settling cells. Transfer these cells to a clean microfuge tube.

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11 Dilute the **10 µl** of cells in **10 µl** of **0.4 Mass Percent Trypan blue** . Transfer **10 µl** of this diluted suspension to a Countess cell counting chamber; allow to sit for **00:00:30** .

Note that this step is performed outside the hood and is NONSTERILE.

12 

Insert the slide into the Countess II automated cell counter. Note the calculated live cell density. You will use this density (not the total density) to calculate the number of cells needed for seeding.

13 Calculate appropriate volume of cells to add to each 6-well.

- Typical seeding number for **IMR90-4 iPSCs** is between **100,000 – 120,000 cells per well**.
- Typical seeding number for **CC3** and **CD12 iPSCs** is **150,000 cells per well**

14 

Resuspend cells in appropriate volume of E8 + **10 Micromolar (µM) ROCK inhibitor** (**2 ml/well**).

If you have excess cells, discard the excess before resuspending.

15 Place plate in incubator, and shake plate quickly back and forth (not swirling) to distribute cells evenly.

16 Approximately 24 hours later (**Day 0**), initiate differentiation:

Protocol for Differentiation of Blood-Brain Barrier Endothelial Cells from Human Pluripotent Stem Cells
by Emma Neal,
Department of Chemical Engineering, Vanderbilt University, Nashville, TN, USA
PREVIEW RUN

16.1 

Note: Cells are seeded for differentiation in E8 medium according to the standardized single cell seeding protocol

On **day 0**, aspirate E8 medium and add **2 ml** of E6 per well.

16.2 

Change medium every day using **2 ml** of E6 per well.

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16.3

At **day 4** of E6 treatment, aspirate and add **2 ml** of EC medium with bFGF (basic fibroblast growth factor) and **10 Micromolar (µM)** RA to each well.

Medium is NOT changed during expansion phase.

16.4 BBB subculturing:
On **day 6**, subculture BBB onto plates and Transwell filters according to the following protocol:

Protocol for Subculture of Differentiated Blood-Brain Barrier Endothelial Cells onto Plates and Filters PREVIEW RUN

by Emma Neal,
Department of Chemical Engineering, Vanderbilt University, Nashville, TN, USA

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