Human Cerebral Microvascular Endothelial Cell Line (hCMEC/D3)

Designation: hCMEC/D3
Catalogue Number: CLU512

Description:

The Human Cerebral Microvascular Endothelial Cell Line (hCMEC/D3) was prepared from cerebral microvessel endothelial cells (CECs) by transduction with lentiviral vectors carrying the SV40 T antigen and human telomerase reverse transcriptase (hTERT). This cell line shows a spindle-shaped, elongated morphology similar to primary cultures of brain endothelial cells and also exhibits contact inhibition at confluence when cultured on collagen type I or IV. In addition, this line expresses a variety of brain endothelial markers, adherence junction (AJ) and tight junction (TJ) proteins as well as functional ABC transporters typical to brain epithelium.

hCMEC/D3 represents a stable, easily grown blood brain barrier (BBB) model cell line. It is ideal for drug uptake and active transport studies, as well as for understanding the brain endothelium response to various human pathogens and inflammatory stimuli.

Cell culture conditions:

Note: All cell culture should be conducted in a cell culture hood, with sterile conditions.
Standard tissue culture materials (plates, pipettes etc.) are suitable.

I. Reagents, Media, Serum, Culture Plates

A. Reagents:

- Cultrex® Rat Collagen I lower viscosity (R&D Systems, Trevigen, #3443-100-01)
- Distilled water (Life Technologies, #15230)
- DMSO: Dimethylsulfoxide (Sigma, #D5879)
- Ethyl Alcohol absolute (Carbo Erba, #414607)
- PBS: Phosphate Buffered Saline -CaCl2-MgCl2 (Life technologies, #14190)
- Trypsin/EDTA (Life Technologies, #25200)

B. Medium:

Culture of the hCMEC/D3 cell line is done in supplemented EBM-2 medium, as described below:

- EBM-2 Endothelial basal medium (Lonza, #190860)
Supplemented with - Fetal Bovine Serum (Gibco, # 12483020)
- Chemically Defined Lipid Concentrate (Life technologies, # 11905031)
- Ascorbic Acid (Sigma, #A4544)
- bFGF: human Basic Fibroblast Growth Factor, (Sigma, #F0291)
- HEPES 1M (Life Technologies, #15630-080)
- Hydrocortisone 1mg (Sigma, #H0135)
- Penicillin, 10 000 units-Streptomycin, 10 000µg.mL^{-1} (Life technologies, #15140-122)

II. Preparation of Media and Solutions

A. Complete Medium preparation:

Complete medium *(final concentration)* EBM-2:

- Fetal Bovine Serum *(5%)*	25 mL
- Penicillin-Streptomycin *(1%)*	5mL
- Hydrocortisone *(1.4µM)*	250µL (2.8mM)
- Acid Ascorbic *(5µg.mL^{-1})*	2.5mL (1mg.mL^{-1})
- Chemically Defined Lipid Concentrate *(1/100)*	5mL
- HEPES *(10mM)*	5mL (1M)
- bFGF *(1ng.mL^{-1})*	2.5 mL (200ng.mL^{-1})
- EBM-2	to 500mL

bFGF aliquots are stored at -20°C and added extemporaneously in the culture medium.

B. Solutions:

- Freezing medium:

Mix 95% fetal bovine serum with 5% DMSO.

- Fetal bovine Serum (Gibco):

Heat inactivation at 56 degrees before use.

- 200 ng.mL^{-1} bFGF solution:

- Dissolve 25µg bFGF in 500 µL PBS to prepare a 50µg.mL^{-1} stock solution.
- Mix 50µL of this stock solution with 12.5 mL of EBM-2 in order to make a 200X bFGF solution.

- 2.8 mM Hydrocortisone solution:

Dissolve 1mg in 1mL Ethyl Alcohol absolute to make a 2000X hydrocortisone solution.

- Thin rat collagen coating:

- Dilute rat collagen in distillated water to get a final protein concentration at 150µg.mL^{-1}.
- Add enough solution to recover the culture surface and incubate at 37°C for at least 1 hour.
- Wash with PBS and replace with culture medium.

III. Cell Maintenance and Culture Procedures

A. Routine Culture

This *in vitro* BBB model consists of the human brain endothelial cell line hCMEC/D3 cultured at 37°C in 5% CO2. Inserts and flasks/Petri dishes are beforehand recovered with the thin collagen I coating.

Cells are passed twice a week. Seed Petri dishes or flasks at a density of 25 000 cells per cm^2. Three-Four days after seeding on flasks or petri dishes, cells reach confluence and can be trypsinised.

Cells can be used until passage 35 without loss of BBB properties. **When purchasing the cell line, you can expect to receive a vial with a passage number between 25 -27.**

B. Thawing Cells

- Cells are rapidly thawed at 37°C and seeded in a 60 mm Petri Dish pre-coated with collagen type I thin coating.
- After 1 or 2 hours, when cells are attached, medium is replaced with fresh medium.
- Cells can be maintained in this dish until they reach confluence.

C. Freezing Cells

- Confluent cells are trypsinised and centrifuged at 1000g for 10 minutes.
- Pellet is then resuspended with freezing medium and frozen at a dilution of 2-3 million cells per cryovial.
- These are placed at -80°C overnight and the next morning in liquid nitrogen.

References:

Weksler, B. B., Subileau, E. A., Perriere, N., et al. (2005). Blood-brain barrier-specific properties of a human adult brain endothelial cell line. *The FASEB journal*, *19*(13), 1872-1874.

Poller, B., Gutmann, H., Krähenbühl, S., et al. (2008). The human brain endothelial cell line hCMEC/D3 as a human blood-brain barrier model for drug transport studies. *Journal of neurochemistry*, *107*(5), 1358-1368.

Weksler, B., Romero, I. A., & Couraud, P. O. (2013). The hCMEC/D3 cell line as a model of the human blood brain barrier. *Fluids Barriers CNS*, *10*(1), 16.

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