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BCRJ Code: 0401

Cell Line: hCMEC/D3

Species: Homo sapiens

Vulgar Name: Human

Tissue: Cerebral microvessel

Morphology: Spindle-shaped, elongated

Disease: Normal

Derivation: The hCMEC/D3 cell line was derived from human temporal lobe microvessels isolated from tissue excised during surgery for control of epilepsy. The primary isolate was enriched in cerebral endothelial cells (CECs). In the first passage, cells were sequentially immortalized by lentiviral vector transduction with the catalytic subunit of human telomerase (hTERT) and SV40 large T antigen, following which CEC were selectively isolated by limited dilution cloning, and clones were extensively characterized for brain endothelial phenotype. This brain microvascular endothelial cell line represents one such model of the human BBB that can be easily grown and is amenable to cellular and molecular studies on pathological and drug transport mechanisms with relevance to the central nervous system (CNS).

Applications: 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.

Biosafety: 2

Additional Info: 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.

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Culture Medium:

EBM-2 Endothelial basal medium (Lonza, #190860) Supplemented with: - Fetal Bovine Serum 5% - Chemically Defined Lipid Concentrate (Life technologies, #11905031): 1/100 - Ascorbic Acid (Sigma, #A4544) - 5µg/mL - bFGF: human Basic Fibroblast Growth Factor, (Sigma, #F0291) - 1ng/mL - HEPES: 10mM - Hydrocortisone (Sigma, #H0135): 1.4µM

Subculturing:

ECM Coating of Flasks: Thaw Collagen Type I, Rat Tail at room temperature. Dilute 1 mL of Collagen Type I with 19 mL 1X PBS. Mix gently. Scale up according to the volumes required. Coat flask with 1:20 diluted Collagen Type I solution. Use 5-10 mL for T75 flasks and 15-25 mL for T225 flasks. Incubate in 37°C incubator for at least one hour before use. Note: Flasks may be coated 5-6 days in advance and stored at 2-8°C in the coating solution. Aspirate the coating solution just before plating the cells. Subculturing: Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of hCMEC/D3 cells. Apply 3-5 mL of trypsin-EDTA solution and incubate in a 37°C incubator for 3-5 minutes. Inspect the plate and ensure the complete detachment of cells by gently tapping the side of the plate with the palm of your hand. Add 8 mL of hCMEC/D3 medium (pre-warmed to 37°C) to the plate. Gently rotate the plate to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells. Discard the supernatant. Apply 2 mL of hCMEC/D3 media (pre-warmed to 37°C) to the conical tube and resuspend the cells thoroughly. Plate the cells to the desired density.

Subculturing Subcultivation Ratio:

Typical split ratio is 1:3 to 1:6.

Culture Conditions:

Atmosphere: air, 95%; carbon dioxide (CO₂), 5% Temperature: 37°C

Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:

Do not thaw the cells until the recommended medium is on hand. Cells are thawed in hCMEC/D3 Medium. Remove the vial of hCMEC/D3 cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells. **IMPORTANT:** Do not vortex the cells. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process. Using a 10 mL pipette, slowly add dropwise 9 mL of hCMEC/D3 Medium (pre-warmed to 37°C) to the 15 mL conical tube. **IMPORTANT:** Do not add the whole volume of media at once to the cells. This may result in decreased cell viability due to osmotic shock. Gently mix the cell suspension by slow pipeting up and down twice. Be careful to not introduce any bubbles. **IMPORTANT:** Do not vortex the cells. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells. Decant as much of the supernatant as possible. Resuspend the cells in a total volume of 10 -12 mL hCMEC/D3 medium (pre-warmed to 37°C). Plate the cell mixture onto a pre-coated T75 tissue culture flask. Incubate the cells at 37°C in a 5% CO₂ humidified incubator. The next day, exchange the medium with fresh hCMEC/D3 Medium pre-warmed to 37°C. Exchange with fresh medium every two to three days thereafter. When the cells are approximately 80% confluent (3-4 days after plating cells at the density they can be dissociated with trypsin-EDTA and passaged or alternatively frozen for later use.

References:

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