

Data Sheet

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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
Addtional 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: - Fe Bovine Serum 5% - Chemically Defined Lipid Concentrate (Life technologies 11905031): 1/100 - Ascorbic Acid (Sigma, #A4544) - 5µg/mL - bFGF: human Fibroblast Growth Factor, (Sigma, #F0291) - 1ng/mL - HEPES: 10mM - Hydrocortisone (Sigma, #H0135): 1.4μM	etal , # Basic
Subculturing:	ECM Coating of Flasks: Thaw Collagen Type I, Rat Tail at room temperature. mL of Collagen Type I with 19 mL 1X PBS. Mix gently. Scale up according to volumes required. Coat flask with 1:20 diluted Collagen Type I solution. Use for T75 flasks and 15-25 mL for T225 flasks. Incubate in 37°C incubator for a one hour before use. Note: Flasks may be coated 5-6 days in advance and s 2-8°C in the coating solution. Aspirate the coating solution just before platin cells. Subculturing: Carefully remove the medium from the T75 tissue cultur containing the confluent layer of hCMEC/D3 cells. Apply 3-5 mL of trypsin-E solution and incubate in a 37°C incubator for 3-5 minutes. Inspect the plate ensure the complete detachment of cells by gently tapping the side of the p the palm of your hand. Add 8 mL of hCMEC/D3 medium (pre-warmed to 37 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 minutes to pellet the cells. Discard the supernatant. Apply 2 mL of hCMEC// (pre-warmed to 37°C) to the conical tube and resuspend the cells thorough the cells to the desired density.	Dilute 1 the 5-10 mL t least tored at ng the re flask DTA and olate with °C) to 3-5 D3 media ly. Plate
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)	

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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 9ml of hCEMC/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:	Couraud PO, et al. (2008) The human brain endothelial cell line hCMEC/D3 as a human blood-brain barrier model for drug transport studies. Fluids Barriers CNS. 2013 Mar 26;10(1):16. Dauchy S, et al. (2009) Expression and transcriptional regulation of ABC transporters and cytochromes P450 in hCMEC/D3 human cerebral microvascular endothelial cells. Biochem Pharmacol. 2009 Mar 1;77(5):897-909. Markoutsa E, et al. (2011) Uptake and permeability studies of BBB-targeting immunoliposomes using the hCMEC/D3 cell line. Eur J Pharm Biopharm. 2011 Feb;77(2):265-74. Couraud PO., et al. (2013) The hCMEC/D3 cell line as a model of the human blood brain barrier. Fluids Barriers CNS. 2013 Mar 26;10(1):16. Coureuil M, et al. (2009) Meningococcal type IV pili recruit the polarity complex to cross the brain endothelium. Science. 2009 Jul 3;325(5936):83-7. Schreibelt G, et al. (2007) Reactive oxygen species alter brain endothelial tight junction dynamics via RhoA, PI3 kinase, and PKB signaling. FASEB J. 2007 Nov;21(13):3666-76. Tai LM, et al. (2009) P-glycoprotein and breast cancer resistance protein restrict apical-to-basolateral permeability of human brain endothelium to amyloid-beta. J Cereb Blood Flow Metab. 2009 Jun;29(6):1079-83. Coureuil M, et al. (2010) Meningococcus Hijacks a β 2-adrenoceptor/ β -Arrestin pathway to cross brain microvasculature endothelium. Cell. 2010 Dec 23;143(7):1149-60. Georgieva JV, et al. (2011) Surface characteristics of nanoparticles determine their intracellular fate in and processing by human blood-brain barrier endothelial cells in vitro. Mol Ther. 2011 Feb;19(2):318-25.

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