Product sheet

hCMEC/D3 Cells | 305024

General information

Description	The HCMEC/D3 cell line represents an immortalized human cerebral microvascular endothelial cell line, extensively utilized in the study of the blood-brain barrier (BBB). This cell line was generated through the transduction of primary human cerebral microvascular endothelial cells with a lentiviral vector expressing human telomerase reverse transcriptase (hTERT), a crucial enzyme for maintaining telomere length and thereby promoting cellular longevity without transforming the cell phenotype. The introduction of hTERT helps these cells to bypass the replicative senescence that limits the lifespan of primary cells, allowing sustained propagation in culture. HCMEC/D3 cells retain key physiological and morphological characteristics of primary cerebral endothelial cells, making them a valuable model for in vitro studies of the BBB. These include the expression of tight junction proteins such as claudin-5, occludin, and zonula occludens-1, which are critical for maintaining barrier integrity. The cells also express various transporters and receptors typical of the cerebral endothelium, supporting their use in studies related to drug delivery and neurovascular disorders. The ability of HCMEC/D3 to form a tight monolayer with high electrical resistance underscores their suitability for BBB permeability assays. Research utilizing HCMEC/D3 cells has covered a wide range of applications, including the investigation of cerebral pathologies such as stroke, multiple sclerosis, and metastasis of cancer to the brain. Their compatibility with various molecular biology techniques also makes them an excellent tool for studying endothelial cell responses to inflammatory stimuli, shear stress, and neurotoxic substances. This cell line provides a robust, reproducible platform for dissecting the molecular events at the cerebral endothelial level, contributing valuable insights into the complexities of neurovascular health and disease.
Organism	Human
Tissue	Brain, temporal lobe, blood microvessel
Disease	Normal cerebral microvascular endothelium (hTERT and SV40-immortalized; blood-brain barrier model; non-tumorigenic)
Metastatic site	Not applicable (normal brain endothelial cell line; not a tumor sample)
Applications	Blood-brain barrier (BBB) research; neuroinflammation; drug CNS delivery and permeability; transendothelial migration; tight junction biology (claudin-5, occludin, ZO-1); neurological disease modeling; shear stress responses; neurotoxicity testing
Synonyms	HCMEC/D3, CMEC/D3, human Cortical Microvessels Endothelial Cells/D3

Characteristics

Age	Adult
Gender	Female

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Ethnicity	Not specified
Morphology	Endothelial-like (cobblestone)
Cell type	Endothelial cell
Growth properties	Adherent

Regulatory Data

Citation	hCMEC/D3 (Cytion catalog number 305024)
Biosafety level	1
NCBI_TaxID	9606
CellosaurusAccession	CVCL_U985
GMO Status	GMO-S1: This human microvascular endothelial cell line (hCMEC/D3) contains lentiviral constructs encoding SV40 T-Antigen or hTERT, supporting stable immortalization. The insert is integrated into primary endothelial cells. This classification applies only within Germany and may differ elsewhere.

Biomolecular Data

Viruses	Transformant: Simian virus 40 (SV40)

Handling

Culture Medium	EGM -2 MV Microvascular Endothelial Cell Growth Medium-2 BulletKit (from Lonza, Lonza catalog number CC-3202)
Supplements	Supplement the supplied EBM-2 Basal Medium as recommended by the manufacturer
Dissociation Reagent	Accutase or 0.25% Trypsin-EDTA (brief; do not over-trypsinize)
Doubling time	approx. 24 to 36 hours
Subculturing	Remove medium, wash with PBS without Ca^{2+}/Mg^{2+}, add Accutase (3–5 min at 37°C), neutralize with complete medium, centrifuge 300×g 5 min, reseed at 1–2 × 10^4 cells/cm^2 onto collagen-coated flasks.

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Split ratio	1 to 3
Seeding density	1 to 2 × 10^4 cells/cm^2 (on collagen I-coated surfaces)
Fluid renewal	Every 1 to 2 days
Freeze medium	As a cryopreservation medium, we use 50% basal medium + 40% FBS + 10% DMSO, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

Incubation Atmosphere	37°C, 5% CO_2, humidified atmosphere.

Shipping Conditions	Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately −78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.
Storage Conditions	For long-term preservation, place vials in vapor-phase liquid nitrogen at about −150 to −196 °C. Storage at −80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

Quality Control & Molecular Analysis

Sterility	Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods. To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.