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cell based solutions

IMMORTALIZED CELL LINES

P10863-IM

Immortalized Human Corneal Endothelial Cells provided by Innoprot have been obtained by immortalizing human corneal endothelial cells with Lenti-SV40 lentivirus.

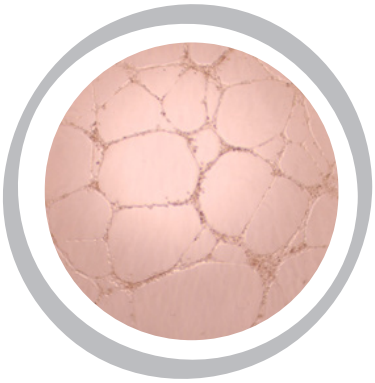
Corneal endothelial cells form a monolayer on the inner surface of the cornea, maintaining its transparency by regulating stromal hydration. Unlike other corneal cells, they have limited proliferative capacity in vivo, making them susceptible to degeneration and injury. Beyond their role in corneal physiology, corneal endothelial cells have been implicated in the regulation of ocular angiogenesis.

In pathological conditions such as Fuchs' endothelial dystrophy or surgical trauma, endothelial cell loss leads to corneal edema and vision impairment. Additionally, these cells play a role in ocular angiogenesis, as their dysfunction may influence aberrant vascularization processes, including retinal neovascularization, through pathways like VEGF signaling.

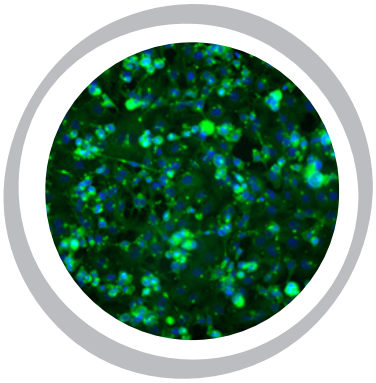
IMMORTALIZED HUMAN
CORNEAL ENDOTHELIAL CELLS

Product Type: Immortalized Human Corneal Endothelial Cells
Catalog Number: P10863-IM
Immortalization: SV40 Large T Antigen. G418 resistant.
Number of cells: >1x10^6 cells (cryopreserved vials)
Storage: Liquid Nitrogen
Recommended Medium: Endothelial Medium Kit (Ref: P60104)
Product Characterization: Expression of constitutive phenotypic endothelial markers (CD31 and Von Willebrand factor). Functional analysis (angiogenesis reorganization).

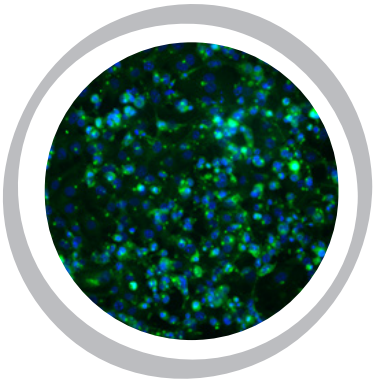
Phase-contrast



Tubule formation



CD-31



Von-Willebrand

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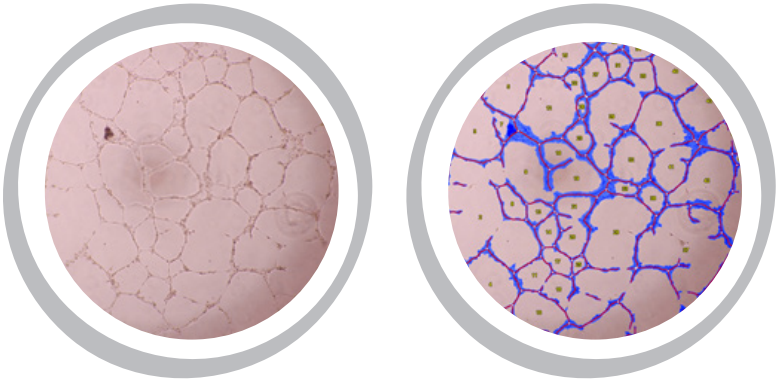
About Immortalized Human Corneal Endothelial Cells

The immortalized human corneal endothelial cell line has been developed through genetic modification of primary culture of human corneal endothelial cells, employing the SV40LT protein as the immortalization method. The SV40LT protein, derived from the Simian Virus 40 Large T-antigen, has been introduced to confer immortality to the cells, allowing for an extended lifespan compared to primary cells that typically undergo senescence after a limited number of passages. The use of SV40LT for immortalization is a common technique in cell biology and allows for the establishment of cell lines with a more stable and prolonged growth capacity. This modification enables researchers to conduct long-term experiments and studies that require consistent cellular behavior over an extended period. Primary cells exhibit senescence following the 5th passage, whereas the SV40LT-transduced cells demonstrate a prolonged viability, extending beyond 20 passages.

Functional Analysis: Angiogenesis Reorganization

This assay evaluates the ability of endothelial cells, seeded on Matrigel-coated plates, to form capillary-like structures (also known as tubules). It is widely used to assess the effects of various compounds on promoting or inhibiting tube formation, providing valuable insights into angiogenesis regulation. For this assay, 18,000–25,000 cells per well are seeded in 96-well plates pre-coated with Matrigel. Tubule formation is observed within 24 hours.



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Culturing conditions

1 IMMEDIATELY UPON DELIVERY

1.1 Remove the vial from the shipping container to check for freezing.

1.2 Transfer the frozen vial to liquid nitrogen until ready to thaw.

2 THAWING CELLS:

2.1 Fibronectin coating step (optional): Prepare a fibronectin coated flask (2 µg/cm2) is recommended depending on the type of flasks you are using (i.e. if not using pre-treated flasks for endothelial cells). Add 3 ml of sterile Dulbecco's phosphate buffered saline (DPBS) to a T-25 flask and then add 50 µl of fibronectin stock solution (1 mg/ml, Innoprot cat. no. P8248). Leave the flask in incubator overnight. Aspirate fibronectin solution and add 15 ml of complete medium to the culture vessel. The fibronectin solution can be used twice. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.

2.2 Prepare "Thawing medium" by combining 500 ml of basal medium, 25 ml of fetal bovine serum, 5 ml of Growth supplement and 5 ml of penicillin/streptomycin solution.

2.3 Thaw cells rapidly in a 37ºC water bath; avoid allowing the sample to warm to 37ºC. Cryovials should be cool to the touch when removed.

2.4 Remove the vial, wipe it dry, and transfer it to a sterile field.

2.5 Rinse the vial with 70% ethanol, then wipe to remove excess. Open the vial and resuspend its contents using a 1 ml Eppendorf pipette.

2.6 Dispense the contents into a 25 cm^2 culture flask with warm complete media (FBS percentage can be increased up to 10% for better culture establishment).

2.7 Place the flask in the incubator.

2.8 For optimal results, avoid disturbing the culture for 16 hours after initiation. Change the growth medium the next day to remove unattached cells, then every other day thereafter.

3 MAINTENANCE OF THE CULTURE:

3.1 Change medium 48 hours after establishing a subculture.

3.2 Subculture when cells are over 90% confluent.

4 SUBCULTURING:

Remove medium, rinse with 0.05% trypsin-EDTA solution. Add 1 to 2 mL of trypsin-EDTA solution and allow the flask to sit until cells detach. Add fresh culture medium, aspirate, and dispense into new culture flasks. Recommended subcultivation ratio of 1:2 to 1:3.

Medium Renewal: 2 to 3 times per week.

Reagents for cryopreservation: Cryostor S10.

Quality Control / Biosafety

The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi.

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