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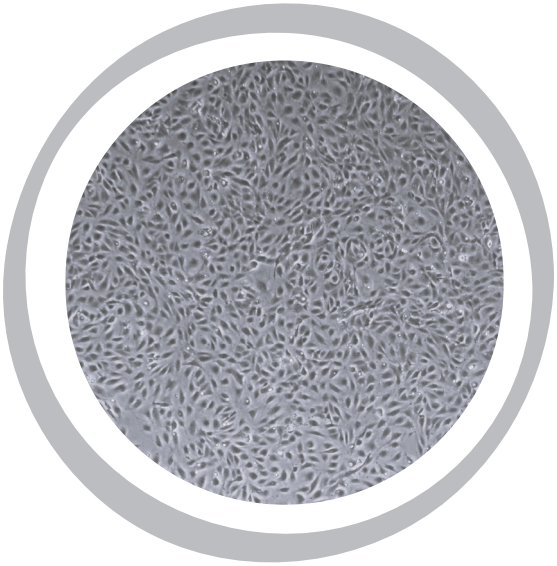
IMMORTALIZED CELL LINES

IMMORTALIZED HUMAN
SPLENIC ENDOTHELIAL CELLS

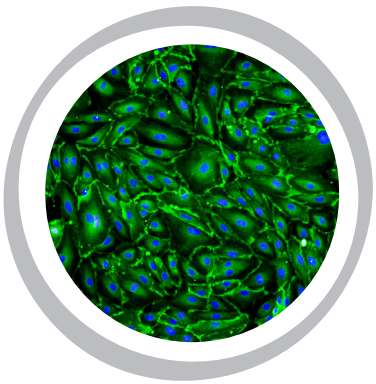
Product Type: Immortalized Human Splenic Endothelial Cells
Catalog Number: P10691-IM
Immortalization: SV40T + BMi-E7. G418 and Puromycin resistant.
Number of cells: >1x10^6 cells (cryopreserved vials)
Storage: Liquid Nitrogen
Recommended Medium: Endothelial Cell Medium Kit (Ref: P60104)
Product Characterization: Positive for vWF/Factor VIII, CD31 (P-CAM) and tubule formation.

P10691-IM

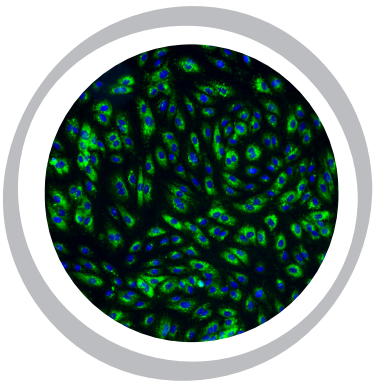
Human splenic endothelial cells are specialized cells lining the vasculature of the spleen, where they play a pivotal role in regulating blood filtration, immune cell trafficking, and the removal of senescent or damaged erythrocytes. These cells form part of the unique splenic microenvironment, contributing to both vascular homeostasis and immune surveillance by facilitating interactions between circulating blood components and resident immune cells. They are actively involved in antigen capture, leukocyte recruitment, and the regulation of the splenic red and white pulp interface. In pathological conditions such as splenomegaly, chronic inflammation, or hematologic malignancies, splenic endothelial dysfunction can disrupt blood flow regulation and impair immune responses. Furthermore, their capacity to respond to inflammatory mediators and hemodynamic changes is essential for understanding vascular and immunological disorders affecting the spleen. Immortalized human splenic endothelial cells provide a robust in vitro model for investigating splenic vascular biology, immune–vascular interactions, and the molecular mechanisms underlying both physiological and disease-associated processes.



Phase-contrast



CD31



VWF

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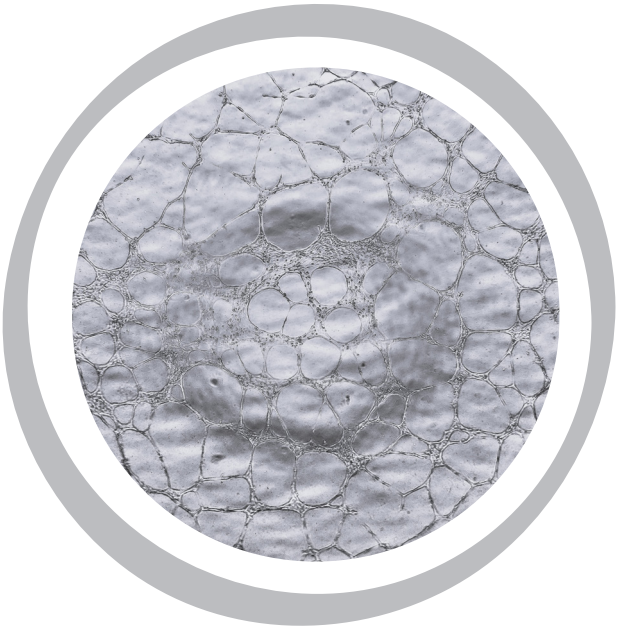
About Immortalized Human Splenic Endothelial Cells

The immortalized human splenic endothelial cell line has been established through genetic modification of primary human splenic endothelial cells using a combination of the SV40 large T antigen (SV40T) and BMI1/E7 as immortalizing agents. SV40T, derived from the Simian Virus 40 Large T-antigen, disrupts key regulators of the cell cycle, while BMI1/E7 enhances proliferative capacity by suppressing senescence-associated pathways and supporting telomere maintenance. Together, these factors enable the cells to bypass replicative senescence and achieve extended proliferative potential. This dual-factor approach results in a stable cell line with robust long-term growth characteristics, suitable for repeated passaging and consistent experimental use. While primary human splenic endothelial cells typically lose proliferative capacity after approximately five passages, SV40T and BMI1/E7–transduced cells maintain viability and expansion potential beyond 20 passages. This makes them a reliable tool for in vitro studies focused on cardiovascular endothelial biology, microvascular dysfunction, and therapeutic screening.

Functional Assay: Tube Formation

To evaluate the angiogenic functionality of the immortalized cell line, a tube formation assay was performed. Cells were seeded into Geltrex-coated 96-well plates at a density of 18×10^3 cells per well. This extracellular matrix surrogate supports the organization of endothelial cells into capillary-like structures. After 24 hours of incubation at 37°C in a humidified atmosphere with 5% CO_2, the tube formation was verified.



Tubule formation

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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, T-75 flask) is recommended depending on the type of flasks you are using (i.e. no pre-treated flasks for endothelial cells). Add 10 ml of sterile Dulbecco's phosphate buffered saline (DPBS) to a T-75 flask and then add 150 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 1:6.

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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