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cell based solutions

IMMORTALIZED CELL LINES

P10456-IM

Smooth muscle cell (SMC) is the cellular substate of most significant arterial disease. The increased growth potential of vascular SMC represents one of the crucial anomalies responsible for the development of essential vascular diseases.

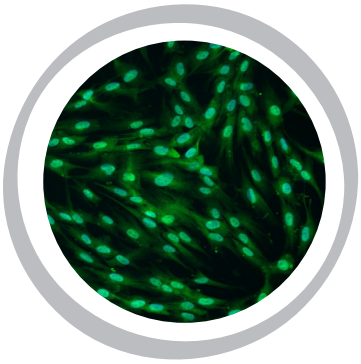
New studies demonstrate that SMC express calcium channels and the expression of ICAM-1 and VCAM-1 on SMC may contribute to the inflammatory reaction in the vascular wall and may actively be involved in the progression and stability of vascular disease.

In vitro culture of human vascular SMC as a model of vascular research played a critical role and continues providing information in the pharmacology and the therapy of vascular diseases.

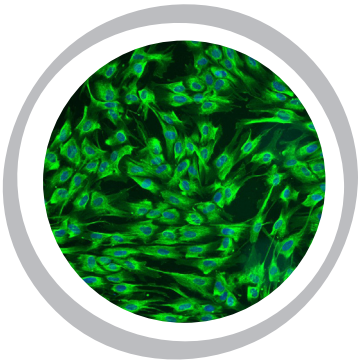
IMMORTALIZED HUMAN

AORTIC SMOOTH MUSCLE CELLS

Product Type: I Immortalized Human Aortic Smooth Muscle Cells
Catalog Number: P10456-IM
Immortalization: SV40 Large T Antigen
Number of cells: >1x10^6 cells (cryopreserved vials)
Storage: Liquid Nitrogen
Source: Human Aorta
Recommended Medium: Smooth Muscle Cell Medium-PLUS (Reference: P60125-PLUS).
Product Characterization: Immunofluorescent method for α-smooth muscle actin and Vimentin.



α-smooth muscle actin



Vimentin

About Immortalized Human Aortic Smooth Muscle Cells

The Immortalized Human Aortic Smooth Muscle Cells has been developed through genetic modification of human primary aortic smooth muscle 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 3rd passage, whereas the SV40LT-transduced cells demonstrate a prolonged viability, extending beyond 30 passages.

IMMORTALIZED CELL LINES

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 Prepare complete medium. Decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.

2.2 Add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.

2.3 Place the vial in a 37ºC waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using a 1 ml eppendorf pipette gently resuspend the contents of the vial.

2.4 Dispense the contents of the vial into the equilibrated culture vessels. A seeding density higher than 7,500 cells/cm^2 is recommended.

Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture.

2.5 Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.

2.7 Return the culture vessels to the incubator.

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

3 MAINTENANCE OF THE CULTURE:

3.1 Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells.

3.2 Change the medium every three days thereafter, until the culture is approximately 70% confluent. Once the culture reaches 70% confluency, change medium every other day until the culture is approximately 90% confluent.

3.3 Subculture when cells are over 90% confluent.

4 SUBCULTURING:

4.1Warm complete medium, trypsin/EDTA solution (T/E Solution), T/E neutralization solution (TNS), and DPBS (Ca++ -and Mg++-free) to room temperature. We do not recommend warming reagents and medium in a 37ºC water bath prior to use.

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Culturing conditions

4.2 Rinse the cells with DPBS. Add 8 ml of DPBS first and then 2 ml of trypsin/EDTA solution into flask (in the case of T-75 flask); gently rock the flask to make sure cells are covered by trypsin/EDTA solution; incubate the flask at 37ºC incubator for 1- 2 minutes or until cells are completely rounded up (monitored with inverted microscope).

4.3 During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS).

4.4 Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37ºC for another 1 to 2 minutes (no solution in the flask at this moment).

4.5 At the end of trypsinisation, one hand hold one side of flask and the other hand gently tap the other side of the flask to detach cells from attachment; check the flask under inverted microscope to make sure all cells are detached.

4.6 Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Add another 5 ml of TNS to harvest the residual cells and transfer it to the 50 ml centrifuge tube.

4.7 Examine the flask under inverted microscope to make sure the cell harvesting is successful by looking at the number of cells left behind. There should be less than 5%.

4.8 Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Resuspend cells in growth medium. Count and plate cells in a new flask with cell density as recommended.

Quality Control / Biosafety

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

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