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IMMORTALIZED CELL LINES

P10472-IM

Human pancreatic stromal cells (IM-hPSCs) are a type of mesenchymal stem/stromal cell (MSC) capable of expansion and appear to naturally and efficiently differentiate into clusters of mature pancreatic cells.

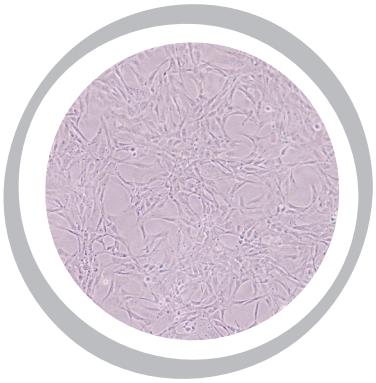
IM-hPSCs displays a mesenchymal character and express lineage-specific marker genes upon induction toward pancreatic endocrine and mesenchymal pathways of differentiation.

Pancreatic islet beta-cell replenishment can be driven by epithelial cells from exocrine pancreas via epithelial-mesenchymal transition and the reverse process MET, while specified pancreatic mesenchymal cells control islet cell development and maintenance.

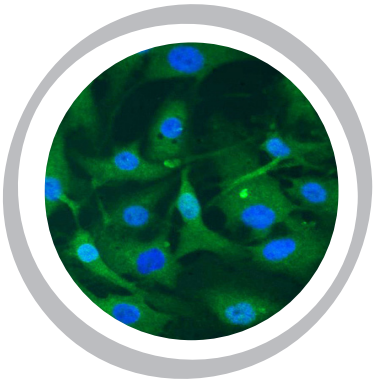
IMMORTALIZED HUMAN

PANCREATIC STROMAL CELLS

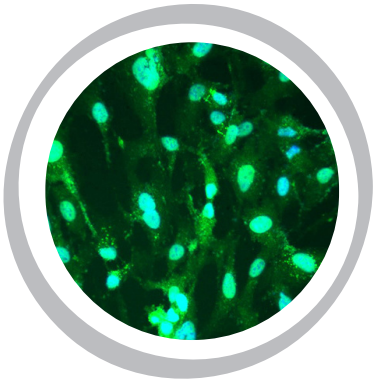
Product Type: Immortalized Pancreatic Stromal Cells
Catalog Number: P10472-IM
Immortalization: SV40 Large T Antigen
Number of cells: >1x10^6 cells (cryopreserved vials)
Storage: Liquid Nitrogen
Source: Human Pancreas
Recommended Medium: Mesenchymal Stem Cell Medium (Reference: P60115).
Product Characterization: Immunofluorescent method for CD56, CD90 and Fibronectin.



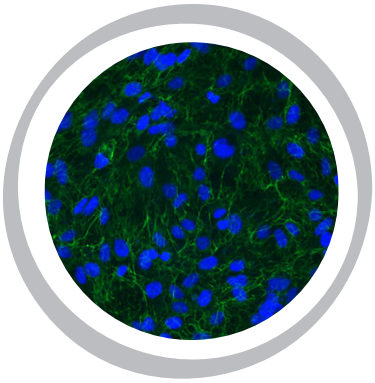
Phase-contrast



CD90



CD56



Fibronectin

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About Immortalized Human Pancreatic Stromal Cells

The Immortalized Human Pancreatic Stromal Cell Line has been developed through genetic modification of a primary culture of human pancreatic stromal 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.

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 Use or prepare a collagen coated flasks (one T-75 flask or two T-25 flasks are recommended) following provider's instructions. Decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. 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.

2.2 Add complete medium to the flask/s. Leave the flask in the hood and go to thaw the cells.

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. Using a 1 ml eppendorf pipette gently resuspend the contents of the vial.

2.6 Dispense the contents of the vial into the equilibrated, collagen coated culture vessels. A seeding density of approximately 15,000-20,000 cells/cm2 is recommended to initiate the cell culture.

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

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2.7 It is also important that cells are plated in collagen coated culture vessels that promote cell attachment and growth. Replace the cap or cover of flask, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.

2.8 Return the culture vessels to the incubator.

2.7 For optimal results, avoid disturbing the culture for 16 hours after initiation. Change the growth medium the next day to remove the 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 it reaches 70% confluence, change medium every other day until the culture is approximately 90% confluent.

3.3 Subculture when cells are over 90% confluent.

4 SUBCULTURING:

4.1 Warm medium, trypsin/EDTA solution (T/E Solution), trypsin neutralization solution (TNS), and DPBS to room temperature. We do not recommend warming the reagents and medium at 37º C waterbath prior to use.

Note: DPBS, trypsin/EDTA solution & trypsin neutralization solution (TNS) are included in the "Primary Cells Detach Kit provided by Innoprot (Reference: P60305).

4.2 Rinse the cells with DPBS.

4.3 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 to 2 minutes or until cells are completely rounded up (monitored with inverted microscope).

4.4 During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS); transfer trypsin/EDTA solution from the flask to the 50 ml centrifuge tube (a few percent of cells may detached); continue incubate the flask at 37ºC for 1 minutes (no solution in the flask at this moment); at the end of trypsinization, 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, add 5 ml of trypsin neutralization solution to the flask and transfer detached cells to the 50 ml centrifuge tube; add another 5 ml of TNS to harvest the residue cells and transfer it to the 50 ml centrifuge tube.

4.5 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%.

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4.6 Centrifuge the 50 ml centrifuge tube (harvested cell suspension) at 1000 rpm (Beckman Coulter Allegra 6R centrifuge or similar) for 5 min; re-suspend cells in growth medium.

4.7 Count cells and plate cells in a new, collagen coated 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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