IMMORTALIZED CELL LINES

P10653-IM

HSteC are intralobular connective tissue cells that exhibit myofibroblast-like or lipocyte phenotypes. These cells play a crucial role in the homeostasis of the liver extracellular matrix, as well as in repair, regeneration, fibrosis, and in the control of retinol metabolism, storage, and release.

Following liver injury, HSteC transform into myofibroblast-like cells and become the major source of type I collagen in the fibrotic liver. Beyond these functions, HSteC have been implicated as regulators of hepatic microcirculation through cell contraction and, in disease states, in the pathogenesis of intrahepatic portal hypertension. The proliferation and migration of HSteC, as well as the expression of chemokines, are involved in the pathogenesis of liver inflammation and fibrogenesis.

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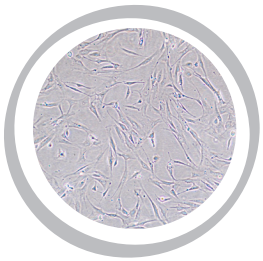
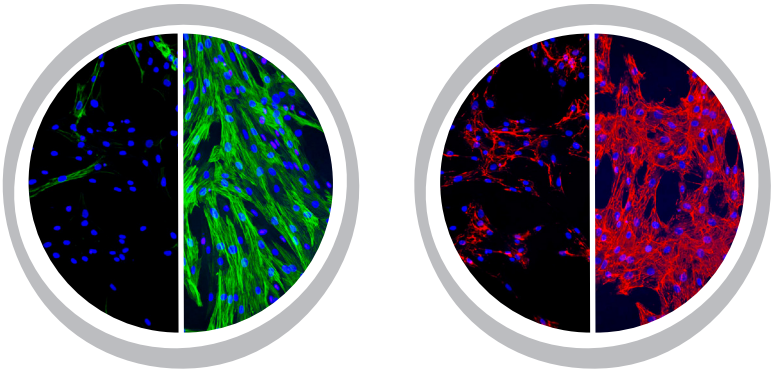
IMMORTALIZED HUMAN HEPATIC STELLATE CELLS



Product Type: Immortalized Human Hepatic Stellate Cells
Catalog Number: P10653-IM
Immortalization: SV40 Large T Antigen. G418 resistant
Number of cells: >1x10^6 cells (cryopreserved vials)
Storage: Liquid Nitrogen
Recommended Medium: Stellate Cell Medium (Reference: P60126)
Product Characterization: Cytochemical staining for αSMA and Fibronectin.

About Immortalized Human Hepatic Stellate Cells

Immortalized human hepatic stellate cells have been obtained by immortalizing human hepatic stellate cells (HSteC) with Lenti-SV40T lentivirus. The SV40LT protein, derived from the Simian Virus 40 Large T-antigen, is used for this immortalization method. The introduction of the SV40LT protein confers immortality to the cells, significantly extending their lifespan compared to primary cells, which typically undergo senescence after a limited number of passages. Using SV40LT for immortalization is a common technique in cell biology, allowing for the establishment of cell lines with more stable and prolonged growth capacity. This modification enables researchers to conduct long-term experiments and studies requiring consistent cellular behavior over an extended period. These immortalized cells were controlled and compared in culture side by side with primary cells. While primary cells enter senescence after the third passage, SV40T-transduced cells surpass 30 passages. Treatment with 5 ng/ml TGF-β for 72 hours results in an increase in the expression of αSMA and Fibronectin.



αSMA basal αSMA TGF-β Fibronectin basal Fibronectin TGF-β

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IMMORTALIZED CELL LINES

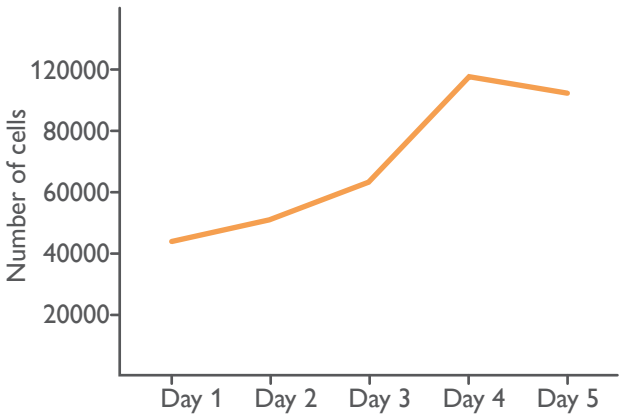
Formation of Mixed Three-Dimensional Cultures

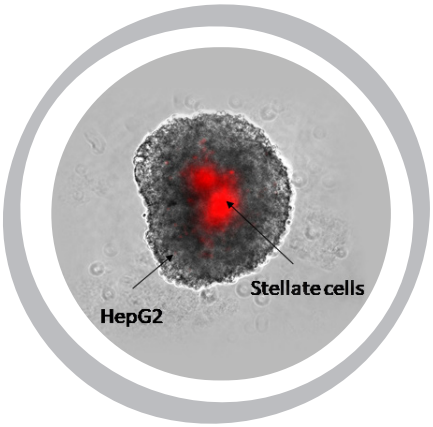
Immortalized human hepatic stellate cells transduced with Red Fluorescent Protein are seeded with HepG2 cells at a ratio of 1:10 in Nunclon Sphera 96-well plates, using DMEM with 10% FBS and 1% Geltrex (100 µl/well). The plate is centrifuged at 250 g for 2 minutes and incubated at 37°C for 13 days.

Culture Progression:

Day 1: Small spheroids with a homogeneous distribution of both cell types are observed.

From Day 3 onwards: The stellate cells are observed to position themselves in the center of the mixed cultures.





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 a Poly-L-Lysine Coated Flask. Use a T-75 flask and coat it with poly-L-lysine (2 µg/cm^2). Add 10 ml of sterile water to the flask, then add 15 µl of poly-L-lysine stock solution. Incubate the flask overnight or for a minimum of one hour at 37°C.
2.2 Prepare complete medium: Decontaminate the external surfaces of the medium and supplements with 70% ethanol, and transfer them to a sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette, rinsing each tube with the medium to recover the entire volume.
2.3 Rinse the poly-L-lysine coated flask twice with sterile water, then add 20 ml of complete medium to the flask. Leave the flask in the hood and proceed to thaw the cells.

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Culturing conditions

2.4 Place the vial in a 37°C water bath, holding and rotating it gently until the contents are completely thawed. Remove the vial immediately, wipe it dry, rinse with 70% ethanol, and transfer it to a sterile field. Remove the cap carefully without touching the interior threads. Gently resuspend the contents with a 1 ml Eppendorf pipette.
2.5 Dispense the vial contents into the equilibrated, poly-L-lysine coated culture vessels, recommending a seeding density of 5,000 cells/cm^2.
Note: Dilution and centrifugation of cells after thawing are not recommended as these actions can be more harmful than the effect of DMSO residue in the culture. It is also important to plate cells in poly-L-lysine coated culture vessels to promote cell attachment and growth.
2.6 Replace the cap or cover of the flask, and gently rock the vessel to distribute the cells evenly. Loosen the cap if necessary to permit gas exchange.
2.7 Return the culture vessels to the incubator.
2.8 For best results, do not disturb the culture for at least 16 hours after initiation. Change the growth 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 morning after establishing the culture from cryopreserved cells.
3.2 Change the medium every three days thereafter until the culture is approximately 70% confluent.
3.3 Once the culture reaches 70% confluence, change the medium every other day until the culture is approximately 90% confluent.

4. SUBCULTURING

4.1 Subculture when the culture reaches 90% confluence or above.
4.2 Prepare poly-L-lysine-coated culture vessels (2 µg/cm^2) one day before subculture.
4.3 Warm 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 37°C water bath prior to use.
Note: DPBS, trypsin/EDTA solution, and trypsin neutralization solution are included in the "Primary Cells Detach Kit" provided by Innoprot (Cat. Nº P60305).
4.4 Rinse the cells with DPBS.
4.5 Add 8 ml of DPBS and then 2 ml of T/E solution to the flask (for a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Incubate at 37°C for 1 to 2 minutes or until cells completely round up. Use a microscope to monitor the change in cell morphology.
4.6 During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS).
4.7 Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percentage of cells may detach) and continue incubating the flask at 37°C for another 1 to 2 minutes (no solution should be in the flask at this time).

Culturing conditions

4.8 At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to ensure all cells are detached.
4.9 Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect residual cells.
4.10 Examine the flask under a microscope to ensure a successful cell harvest, ensuring fewer than 5% of cells are left behind.
4.11 Centrifuge the 50 ml tube at 1000 rpm for 5 minutes. Resuspend cells in culture medium.
4.12 Count and plate cells in a new poly-L-lysine-coated culture vessel at the recommended cell density.

Quality Control / Biosafety

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

Caution: Handling human-derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV, and HCV DNA, diagnostic tests are not necessarily 100% accurate. Proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following universal procedures for handling products of human origin as the minimum precaution against contamination[1].

[1] Grizzle, W. E., & Polt, S. S. (1988). Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. J Tissue Culture Methods, 11(4).

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