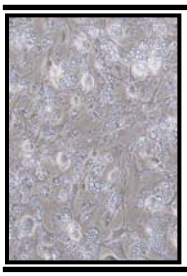
REF: P10623

MOUSE HEPATIC STELLATE CELLS

HEPATIC CELL SYSTEM INNOPROFILE™



Product Type:	Cryo-preserved Stellate Cells
Catalog Number:	P10623-1
Source:	Mouse Liver (Swiss strain)
Number of Cells:	5 x 10^5 Cells / vial (1ml)
Storage:	Liquid Nitrogen

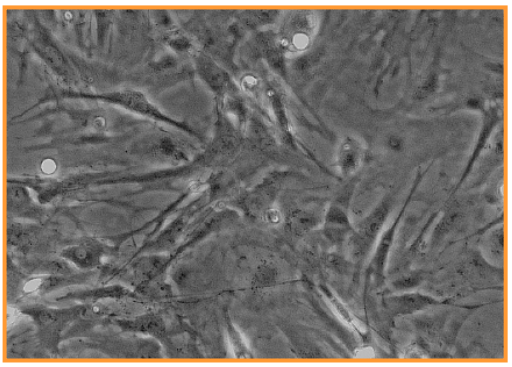
Mouse Hepatic Stellate Cells (MHSteC) provided by Innoprot are isolated by Innoprot from Swiss mice. MHSteC are cryopreserved immediately after purification and delivered frozen (passage "0"). MHSteC is not recommend for expanding or long term cultures since these cells would differentiate to become fibroblat-like cells immediately after plating and they will not proliferate in culture.

MHSteC are intralobular connective tissue cells. They participate in the homeostasis of liver extracellular matrix, repair, regeneration, fibrosis and control retinol metabolism, storage and release. Following liver injury, MHSteC transform into myofibroblast-like cells and are the major source of type I collagen in the fibrotic liver. Beyond these feature, MHSteC have been implicated as regulators of hepatic microcirculation via cell contraction, and in disease states, in the pathogenesis of intrahepatic portal hypertension. MHSteC possess voltage-activated calcium current, express the low affinity nerve growth factor receptor p75, and undergo apoptosis in response to nerve growth factor stimulation.

Recommended Medium

- Stellate Cell Medium

  (Reference: P60126)



Product Characterization

Immunofluorescent method

- Desmin
- α-actin

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

Product Use

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures

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INSTRUCTIONS FOR CULTURING CELLS

IMPORTANT: Cryopreserved cells are very delicate. Thaw the vial in a 37 °C waterbath and return them to culture as quickly as possible with minimal handling!

For proliferating cells:

1. For proliferating cells: Spray the culture vessel (flask, plate or slide) with 70% ethanol for disinfection. Transfer the cells into 37°C, 5% CO_2 incubator and allow equilibrating for 2 hours. After cells have equilibrated, remove shipping medium from the culture vessel and replace with fresh medium.

For Cryopreserved vials. Set up culture after receiving the order:

1. Prepare a poly-L-lysine coated flask (2 µg/cm^2, T-75 flask is recommended) and leave the flask in incubator overnight (minimum one hour at 37°C incubator).

2. 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.

3. Rinse the poly-L-lysine coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.

4. Place the vial in a 37°C 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 1 ml eppendorf pipette gently resuspend the contents of the vial.

5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 20,000 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 DMSO residue in the culture. It is also important that stellate cells are plated in poly-L-lysine coated culture vessels that promote cell attachment.*

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

7. Return the culture vessels to the incubator.

8. For best result, do not disturb the culture for at least 24 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter. A healthy culture will display stellate or spindle-shaped cell morphology with nongranular cytoplasm after two to three days in culture.

Maintenance of Culture:

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

2. Change the medium every two to three days thereafter

It is not recommended that mouse hepatic stellate cells be subcultured beyond their initial plating

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Cell Replating:

If you would like to replate MHSteC into a new culture, please follow the following steps:

1. Prepare laminin or poly-L-lysine coated dishes, coverlips or plates (2 µg/cm^2) one day before replating.

2. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to room temperature. We do not recommend warming the reagents and medium at 37ºC waterbath prior to use.

3. Rinse the culture with DPBS.

4. Incubate stellate cells with 5 ml (in the case of T-25 flask) of DPBS diluted trypsin/EDTA solution (5:1) for one or two minutes (monitored with microscope). Add 5 ml of trypsin neutralization solution to the digestion immediately and gently rock the culture vessel.

5. Harvest and transfer released cells into a 15 ml centrifuge tube. Rinse the flask with another 5 ml of growth medium to collect the residue stellate cells. Examine the flask under microscope to make sure the harvesting is successful by looking at the number of cells left behind. There should be less than 5%.

7. Centrifuge the harvested cell suspension at 1000 rpm for 5 min and resuspend cells in growth medium.

8. Count cells and plate them in a new, laminin-coated flask with cell density as recommended

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

[1]. Grizzle, W. E., and 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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