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HUMAN HEPATIC STELLATE CELLS

Catalog Number LTiHHSC-hm
LTiHHSC-om

Product description:

Hepatic Stellate Cells (HSC) are present between hepatocytes and blood vessels in the liver. HSCs are intralobular connective tissue cells having lipocyte or myofibroblast-like phenotypes. HSCs aid in retinol metabolism, storage, release and also contribute to the regulation, regeneration and repair of the extracellular matrix in the liver. In conditions like persistent alcoholism, Hepatitis B, Hmigrepatitis C, fatty liver disease, obesity, and diabetes, HSC are activated into fibrogenic myofibroblast-like cells, which cause liver fibrosis. In addition to these characteristics, HSC have been linked to the pathophysiology of intrahepatic portal hypertension in disease states and as regulators of hepatic microcirculation via cell contraction. The pathogenesis of liver inflammation and fibrogenesis involves the proliferation and migration of HSCs and release of chemokines. An improved understanding of the molecular regulation of HSC activation will help to treat hepatic fibrosis and may also lower morbidity and mortality in individuals with chronic liver injury.

Characterization: Immunofluorescence of antibodies against α-smooth muscle actin.

Specifications:

Tissue	Healthy human liver tissue
Cell line type	Hepatocyte cells
Description	human Hepatic Stellate Cells (HSCs)
Alternative names	HSC Ito cells Hepatic pericytes Hepatic lipocytes Perisinusoidal cells
Material size	$1x10^6$ HSCs
Application	HSCs play a vital role in hepatic stellate cytokine signalling, retinoid metabolism, fibrogenesis. So, they can be used as a model for research work.
Storage conditions	Immediately transfer cells from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture is needed for experiments.
Product application	This product is for research use only.
QC	Tested negative for Hepatitis A, B, C and HIV 1 and 2 viruses. Tested negative for mycoplasma, endotoxin. Tested negative for bacteria, fungi, yeast.

PROTOCOL FOR SEEDING, MAINTENANCE, SUBCULTURING AND CRYOPRESERVATION OF HUMAN HEPATIC STELLATE PRIMARY CELLS

I. SEEDING OF HUMAN PRIMARY HEPATIC STELLATE CELLS FROM FROZEN AMPULE

MATERIALS:

a. Human Hepatic Stellate Cells growth medium (1X) (Catalog #HSC-GM-500).
b. Human Hepatic Stellate Cells (Catalog #LTiHHSC-om)
c. Human Hepatic Stellate Cell Culture Extracellular Matrix T25 flask (Catalog #EC-TCF25LT)
d. 10 to 1000µl pipette tips
e. 37°C incubator with a humidified atmosphere of 5% CO2
f. 5ml centrifuge tubes
g. Adjustable pipettes (2-10µl, 10-100µl, and 100-1000µl), multichannel pipettes and a pipettor.
h. Antibiotic-antimycotic solution

METHODS:

Note:

- Typically, Human hepatic stellate cells take 24-36 hrs to proliferate.
- Human hepatic stellate cells can undergo 6-8 passaging.

a. Remove Human Hepatic Stellate Cell growth medium from refrigerator and place in 37°C water bath for 15 minutes before starting the protocol.
b. Take the cryovial from the cryopreservation tank and thaw the cells in 37°C water bath, by holding the vial partially submerged and swirling the vial constantly. Ideally, thawing time should be one or two minutes.
a. Once thawed, immediately remove the vial from the water bath and wipe the vial with 70% ethanol. Perform below prescribed procedure using aseptic techniques inside biosafety cabinet (Class II type A2).
c. After thawing the cells make sure to process them immediately. Leaving the cells at room temperature for an extended period, without processing them, will reduce the viability of the cells.
d. Transfer the cells directly into a 5ml sterile centrifuge tube and then bring the total volume to 2 ml using Human hepatic stellate cell growth medium.
e. Centrifuge the cells to a soft pellet at 100g for 5 minutes.

f. Remove the supernatant and resuspend the cells in a total volume of 1ml Human hepatic stellate cell growth medium and add to an ECM-coated T25 flask, having 4ml growth media. Addition of antibiotic-antimycotic solution into the flask is recommended.

   Note: The recommended cell seeding density is $1x10^5$ cells per ml for the initial revival of the cells and then can be adjusted based on the growth conditions of the cells.

g. Place the flask into a humidified tissue culture incubator at 37°C and 5% CO_2.

h. Cells should start to attach after 12 hours and should be completely attached in 24-36 hrs.

II. MAINTENANCE OF HUMAN HEPATIC STELLATE CELLS:

MATERIALS:

a. Human Hepatic Stellate Cells growth medium (1X) (Catalog #HSC-GM-500).
b. 10 to 1000 µl pipette tips
c. 37°C incubator with a humidified atmosphere of 5% CO_2
d. 5ml centrifuge tubes
e. Adjustable pipettes (2-10µl, 10-100µl, and 100-1000µl), multichannel pipettes and a pipettor.
f. Antibiotic-antimycotic solution
g. D-PBS (Ca+ and Mg+ free)

METHODS:

a. Perform the below prescribed procedure in sterilized condition inside biosafety cabinet (Class II type A2).
b. When visual inspection shows a majority of the cells attached (approximately 80%), remove the spent Human Hepatic Stellate cell growth medium and rinse the cell monolayer with 1-2 ml of sterile D-PBS to remove all traces of serum. Replenish the flask with fresh growth medium.
c. Feed cells with fresh cell growth medium at every alternative day and treat with recommended antibiotic-antimycotic solution as per requirement.
d. When the cells have reached 75 -80% confluency, subculture the cells (the suggested splitting ratio is 1:2).

III. SUBCULTURING OF HUMAN HEPATIC STELLATE CELLS

MATERIALS:

a. Human Hepatic Stellate Cells growth medium (1X) (Catalog #HSC-GM-500).
b. Human Hepatic Stellate Cells (Catalog #LTiHHSC-om)

c. Human Hepatic Stellate Cell Culture Extracellular Matrix T25 flask (Catalog #EC-TCF25LT)
d. 10 to 1000 µl pipette tips
e. 37°C incubator with a humidified atmosphere of 5% CO_2
f. 5ml centrifuge tubes
g. Adjustable pipettes (2-10µl, 10-100µl, and 100-1000µl), multichannel pipettes and a pipettor.
h. Antibiotic-antimycotic solution
i. D-PBS (Ca+ and Mg+ free)
j. Trypsin-EDTA solution

METHODS:

a. Perform the below prescribed procedure in sterilized condition inside a biosafety cabinet (Class II type A2).
b. When the cells reach 75 -80% confluency, subculture the cells for expansion.
c. Using a sterile pipette, discard the spent culture media. Any materials and solutions coming into contact with cells should always be disposed of properly.
d. Rinse the cell monolayer with 1-2 ml of sterile D-PBS to remove all traces of serum.
e. Add 1 ml of Trypsin-EDTA solution to the T25 flask and incubate at 37°C for 2 to 3 mins. Check the progress of the enzyme treatment every few minutes on an inverted phase-contrast microscope.
f. Once the cells are detached, add 2 ml of fresh Human Hepatic Stellate Cell growth medium with serum to the T25 flask to inactivate the Trypsin-EDTA solution. Make a homogenate suspension of single cells by vigorous pipetting, ensuring washing off any remaining attached-cells from the bottom of the culture flask.
g. Collect the suspended cells in a sterile 5 ml centrifuge tube and then centrifuge at 100 g for 5 minutes to obtain a soft cell pellet.
h. Resuspend the cell pellet in 1ml of fresh growth media making single cell suspension with gentle pipetting.
i. Count the cells by using a hemocytometer and use trypan blue solution to check cell viability.
j. Plate the cells at $1x10^5$ cells per ml density into fresh ECM-coated flask. Add recommended antibiotic-antimycotic solution into the flask as per requirement.

IV. CRYOPRESERVATION OF CELLS:

MATERIALS:

a. Human Hepatic Stellate Cells growth medium (1X) (Catalog #HSC-GM-500).
b. Human Hepatic Stellate Cells (Catalog #LTiHHSC-om)
c. 10 to 1000 µl pipette tips
d. 37°C incubator with a humidified atmosphere of 5% CO2
e. 5ml centrifuge tubes
f. Adjustable pipettes (2-10µl, 10-100µl, and 100-1000µl), multichannel pipettes and a pipettor.
g. Antibiotic-antimycotic solution
h. Cryovial freezing container (pre-chilled)
i. DMSO
j. D-PBS (Ca+ and Mg+ free)
k. Fetal Bovine Serum
l. Sterile cryogenic vial (standing)
m. Trypsin-EDTA solution

METHODS:

Note: Cells that are healthy and rapidly dividing to be frozen.

Freezing media composition: 10% DMSO + 40% FBS + 50% Growth media.

a. Culture selection and examination: Prior to freezing, the culture should be maintained in an actively growing state (log phase or exponential growth) to ensure optimum health and good recovery. Ideally, the culture medium should be changed 24 hours prior to harvesting.
b. Using sterile pipette, remove and discard the spent culture medium. Any materials and solutions coming into contact with cells should always be disposed of properly.
c. Rinse the cell monolayer with 1-2 ml of sterile 1X PBS solution to remove all traces of serum.
d. Add 1 ml of Trypsin-EDTA solution to the T25 flask, and incubate at 37°C in a 5% CO_2 incubator for 2 to 3 minutes. Check the progress of the enzyme treatment every few minutes on an inverted phase-contrast microscope.
e. Once the cells are detached, add 2 ml of fresh Human Hepatic Stellate Cell growth medium with serum to the T25 flask to inactivate the Trypsin-EDTA solution. Make a homogenate suspension of single cells by vigorous pipetting ensuring washing off any remaining cells from the bottom of the culture flask.

f. Collect the suspended cells in a 5 ml centrifuge tube and then centrifuge at 100 g for 5 minutes to obtain a soft cell pellet.
g. Remove the supernatant and resuspend the cell pellet in 1ml culture media. Count the cells by using a hemocytometer and trypan blue solution to check cell viability. Centrifuge the cell suspension and remove the supernatant.
h. Add the required amount of cell freezing media (10% DMSO + 40% FBS + 50% Growth media) to get the cell count to 1 million cells per ml.
i. Label the appropriate number of plastic cryogenic vials with at least the name of the cell and the date.
j. Add 1 ml of the cell suspension to each of the vials and seal.
k. Freezing cells: Place the cryovials in a freezing container (pre-chilled) at -80°C for overnight. A slow and reproducible cooling rate is very important to ensure good recovery of cultures. A decrease of -1 to -3°C / minute is recommended.
l. Next day, transfer the cryovials into a vapor phase of liquid nitrogen condition.