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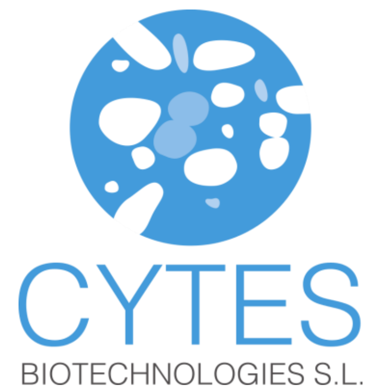
HUMAN PRIMARY
HEPATIC STELLATE CELLS CULTURE



2020.V1

PRODUCT DETAILS

Tissue	Human liver
Package Size	≥100.000 c/vial
Shipped	Cryopreserved
Storage	Liquid Nitrogen
Growth Properties	Adherent
Media	Hepatic stellate cell culture (HSGM)

PROCEDURE

The aim of the present method is to describe the process of culturing human cryopreserved hepatic stellate cells.

Please read through this entire protocol before attempting this procedure.

Required and recommended reagents and consumables

- Thawing and culture media (references)
  - **MHT-NPC**: Thawing media for HSC
  - **HSGM**: Growth media for stellate cells
- Cell culture plates

1. Arrival of the cryopreserved cells in your facilities

Store the cryogenic hepatic stellate vials immediately in vapor phase nitrogen upon receipt.

2. Thawing and culture protocol for cryopreserved primary human hepatic stellate cells

Note: Handle gently and quickly to maintain viability.

Thawing of primary human hepatic stellate

1. Before starting the thawing process, HSGM and HSGM have to be warmed at 37 °C in a water bath.
2. Remove the cryopreserved hepatic stellate cells from the liquid nitrogen and immediately place it in a 37 °C water bath, hold and rotate the vial gently until the contents are completely thawed.

3. Remove the vial from the water bath, wipe dry, rinse the cryovial with 70% ethanol for disinfection and transfer it into the sterile flow hood.

4. Using a pipette, gently transfer the cell suspension contained in the cryovial into a sterile 15 mL conical tube with 3 ml preheated HSGM.

5. Add 1 ml of HSGM to the cryovial, in order to recover the cells that have remained inside each cryovial. Then, combine it with the cells in the 15 mL conical tube to ensure that all stellate cells have been transferred.

6. Centrifuge the tube at 250g for 5 min.

7. After centrifugation, aspirate the supernatant and re-suspend the cell pellet in 1-3 mL of fresh HSGM by gently agitating the bottom of the tube.

8. Perform a cell count (see the next section: determination of viability and number of cells)

Determination of viability and number of cells

To determine viability and number of cells from the stellate cells' suspension in step 8, please follow these instructions:

- Carefully homogenize the cell suspension (do not vortex).
- Take 20 µL of the cell suspension and mix it in a 1.5 mL tube with 20 µL of PBS, and 20 µL of Trypan Blue solution (1/3 dilution), and keep the rest of the cell suspension on ice in the laminar flow hood until seeding.
- Gently homogenize the solution by pipetting up/down.
- Add an aliquot of 20 µL with the Trypan Blue/cell mixture into a cell counting chamber.
- Proceed with cell observation and counting under the microscope. Dead cells will appear in blue. In order to determine viability and cell number, count the living and dead cells and use these formulas:

Viability (%) = (Live cell count/Live cell count + dead cell count) x 100

Total Cell number = Viable cell count/Quadrants counted x Dilution factor x 10^4* x Current volume(mL)

**This factor is applicable when it is used a Hemocytometer*

- After determining cell viability and total number of cells, proceed with cell seeding as follows

Instructions for seeding human hepatic stellate cells:

1. After counting the cells, seed them at a cell density of 4.000 cells/ cm^2 in the desired cell culture plates with HSGM.
   Note: Do not use cell culture flasks, because HSCs will become activated faster than culturing in dishes.

2. Once the plates have been seeded shake vigorously in 8 form, except the 96-well plates that should NOT be shaken.

3. Insert the cells in the incubator at 37 °C / 5% CO_2.

4. Do not disturb the culture for at least 16 hours after seeding.

Maintenance of the hepatic stellate cell culture:

- Refresh growth medium the next morning after establishing a culture from cryopreserved cells to remove unattached cells.

- Change the medium every other day thereafter.

- Subculture the cells when the culture reaches around 90% confluency.

Subculture human hepatic stellate cells:

Required and recommended reagents and consumables

- Culture media **HSGM**: Growth media for stellate cells
- Trypsin/EDTA Solution
- Dulbecco's Phosphate Buffered Saline, without Calcium & Magnesium (DPBS)
- Cell culture plates

1. Before starting the subculture protocol, HSGM has to be warmed at 37 °C in a water bath and the trypsin solution, and Dulbecco's Phosphate Buffered Saline, without Calcium & Magnesium (DPBS) at room temperature.

2. Aspirate the MHG-SC, then rinse the monolayer of cells with DPBS and remove the solution by aspiration.

3. Add trypsin solution into the cell culture dish, rock it gently to ensure the solution covers all the cells and incubate in a 37˚C incubator until the cells detach. To monitor the trypsinization progress, use an inverted microscope until cells become rounded. It usually takes about 3 to 6 minutes.

Note: To improve the trypsinization, release the rounded cells from the culture surface by hitting the side of the flask against your palm until most of the cells are detached

4. At the end of trypsinization, wash the cells with MHG-SC by using the same amount of medium used for trypsin

5. Transfer the cell suspension from the cell culture dish to a sterile centrifuge tube and centrifuge at 250xg for 5 minutes.

6. After centrifugation, aspirate the supernatant, re-suspend in 1-2 mL of fresh medium and count cells for seeding (see section: determination of viability and number of cells).

7. Seed the cells at a density of 4,000 cells/cm^2 on collagen I coated plates.
   Note: For rapid growth, you can increase the cell density per cm^2.

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