CYTES BIOTECHNOLOGIES | **Parc Científic de Barcelona (PCB)**
C/Baldiri Reixac 4-8, Tower I, 6th floor| info@cytesbiotech.com
www.cytesbiotechnologies.com | Phone: +34 934 034 553

PRIMARY HUMAN
HEPATIC KUPFFER CELLS CULTURE

CYTES
BIOTECHNOLOGIES S.L.

PRODUCT DETAILS

Tissue	Human liver
Shipped	Cryopreserved
Storage	Liquid Nitrogen
Growth Properties	Adherent

PROCEDURE

The aim of the present method is to describe the process of thawing and culturing of primary human Kupffer cells.

Please read through this entire protocol before attempting this procedure.

Required and recommended reagents and consumables

- Thawing and culture protocol
  - **MNT:** NPCs Thawing Medium
  - **MCK:** Kupffer Cells Culture Medium
  - **Cell culture plates**

1. Arrival of the cryopreserved cells in your facilities

Store the cryogenic Kupffer cells immediately in vapor phase nitrogen upon receipt.

2. Thawing and culture protocol for cryopreserved primary Kupffer cells

Note: Handle gently and quickly to maintain viability.

Thawing of primary human Kupffer cells

1. Remove the cryopreserved Kupffer 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 partially thawed.

2. 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.

3. Using a pipette, gently transfer the cell suspension contained in the cryovial into a sterile 15 ml conical tube with 5 ml of cold MNT. **Note:** Keep samples on ice during the protocol.

4. Add 1 ml of cold MNT 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 Kupffer cells have been transferred and gently invert the conical tube 2-3 times.

5. Centrifuge the tube at 650g for 10min at 4°C.

6. After centrifugation, aspirate the supernatant and resuspend the cell pellet in 1-5 ml of fresh MCK by gently agitating the bottom of the tube.

7. 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 Kupffer cells' suspension in step 7, 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 primary human Kupffer cells:

1. After counting the cells, seed them at a cell density of 100.000-150.000 cells/cm2 in the desired uncoated cell culture plates with MCK.

2. Once the plate 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. Do not disturb the culture for at least 5 days after seeding. **Note:** Please note that human Kupffer cells do not proliferate in culture and cannot be passaged.

Cell culture maintenance procedure:

1. After 5 days in culture, aspirate and replace MCK every other day or as required by the experiment.

2. Continue this schedule until the end of your experiment. **Note:** Drug testing experiments can begin after the Kupffer cells have been in culture for 24-48 hours even though the cells remain in suspension. Cells will begin to attach at approximately day 5, and this point the medium can be refreshed.