

CI-muHepa

Cat. No.: INS-CI-1012



Biosafety Level
Level 1



Storage
vapor phase of liquid nitrogen



Culture Conditions
HCM (Lonza; CC-3198) + 5% FBS
Collagen solution (INS-SU-1017)

General Information

CI-muHepa is a murine hepatocyte cell line. The cell line was immortalized using the [CI-SCREEN](#) technology ([Lipps et al. 2018; Nat Comm](#)).

Organism: Mus musculus (BALB/c)

Tissue: liver

Growth properties: adherent

Cell culture media and reagents

i InSCREENeX recommends Lonza HCM Hepatocyte Culture Medium supplemented with 5% Serum for culture of CI-muHepa cells.

Product	Cat. No.	Volume
HCM Bullet Kit	Lonza #CC-3198	500ml
Fetal bovine serum (FBS)	any standard cell culture-grade FBS	26ml
Collagen solution	INS-SU-1017-50ml	50ml
	INS-SU-1017-300ml	300ml
Freezing medium	INS-SU-1004	30ml

Intended Use

This product is intended for in vitro research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Quality control

Each vial contains $\geq 5 \times 10^5$ cells. Viability is $\geq 80\%$. Cells are negative for mycoplasma contamination.

Upon arrival

Cells are routinely shipped on dry ice. Check all containers for leakage and breakage. Check if cells arrived frozen. After arrival, store the cryopreserved cells in liquid nitrogen vapor, or seed them immediately (please see page 2).

Note: Cells may be stored at -80°C for short periods (<2 days), but this results in reduced viability and irreversible cell damage.

Medium storage and preparation

Please store and prepare HCM cell culture medium according to manufacturer's instructions (Lonza; [#CC-3198](#)). Please store Fetal bovine serum (FBS) according to manufacturer's instructions.

Material:

- HCM Hepatocyte Culture Medium BulletKit Medium (CC-3198). Includes 500ml HBM Basal Medium (CC-3199) and HCM SingleQuot Supplements (CC-4182).
- Fetal bovine serum (FBS). Please use any standard cell culture-grade FBS.

Storage:

- Store Basal Medium at $4-8^\circ\text{C}$.
- Store Supplements at -20°C .
- Store FBS according to manufacturer's instructions.
- Store completed medium (Basal Medium plus Supplements plus 5% FBS) at $4-8^\circ\text{C}$. Completed medium is stable for at least 1 month at $4-8^\circ\text{C}$.

Protocol:

- 1) Thaw Supplements at 15-25°C.
- 2) If stored frozen, thaw FBS at 15-25°C.
- 3) Add 26ml FBS to 500ml HBM Basal Medium for a final FBS concentration of 5% (v/v).
- 4) Add all HCM Singlequots Supplements to 500ml HBM Basal Medium plus 5% FBS. This is the **complete medium**, complete medium is stable for at least 1 month at 4-8°C.

Collagen coating of cell culture vessels

Material:

- Cell culture vessel(s)
- PBS
- Collagen solution (INS-SU-1017)

Protocol:

- 1) Cover the cell culture vessel with coating solution (see table below for the required volume).
- 2) Incubate the cell culture dish for at least 2h (up to overnight) at 37°C in the incubator.
- 3) Aspirate coating solution.
- 4) Wash once with PBS and aspirate PBS.

Vessel	Surface area (cm ²)	Volume (ml)
T75	75	2.5
T25	25	1.4
6well	9.6	0.7
12well	3.5	0.25
24 well	1.9	0.1
96well	0.32	0.05

Note: Coated cell culture vessels may be stored sealed at 2-8°C for up to 7 days.

Recover cryopreserved cells

Do not thaw the cells until the recommended medium and coated flasks are on hand. For initial recovery (after delivery), we recommend thawing the cells on a T25 flask.

Material:

- complete medium
- 15ml tube
- Collagen-coated culture vessel

Protocol:

- 1) Add 4ml pre-warmed medium to a 15ml tube.
- 2) Quickly thaw the cryovial at 37°C in a water bath until only a few ice crystals are visible. Disinfect vial briefly with 70% Ethanol.
- 3) Transfer thawed cell suspension to the 15ml tube containing 4ml medium. Avoid excessive pipetting up and down.
- 4) Centrifuge cells at 200×g for 4min.
- 5) Aspirate supernatant.
- 6) Gently resuspend the cell pellet in complete medium.
- 7) Transfer cells in coated cell culture vessel and place in the incubator (37°C, 5% CO₂).
- 8) Change the medium after 2 days.

Routine Subculture

Change medium every 2-3 days and split the cells at 70-90% confluence. The split ratio after recovery from cryopreservation should not exceed 1:2. For routine maintenance, split ratio can be increased to 1:5 to 1:10.

Material:

- complete medium
- PBS
- Trypsin/EDTA solution (TE)
- Collagen-coated culture vessel

Protocol:

- 1) Aspirate medium.
- 2) Wash with PBS and aspirate PBS.
- 3) Add Trypsin/EDTA (TE) solution to the cells and incubate at room temperature or 37°C for 5-10min, or until the cells detach.
- 4) Examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.
- 5) Resuspend cells in complete medium thereby inactivating the Trypsin/EDTA (TE) solution.
- 6) Transfer an aliquot of the cell suspension to a new coated cell culture vessel containing fresh complete medium.
- 7) Incubate at 37°C and 5% CO₂.

Vessel	Medium or PBS (ml)	TE (ml)
T75 flask	8-10	2
T25 flask	4-5	1
6well	1.5-2	0.5
12well	1	0.2
24 well	0.5	0.1
96well	0.1	0.05

Cryopreservation

Cell should be grown to 90% confluence before cryopreservation. Avoid full confluence before cryopreservation.

Material:

- Freezing medium (INS-SU-1004)
- PBS
- Trypsin/EDTA solution (TE)
- 2% FBS in PBS
- 15ml tube
- cryovial(s)
- freezing container ("Mr. Frosty" or similar)

Protocol:

- 1) Aspirate medium.
- 2) Wash with PBS and aspirate PBS.
- 3) Add Trypsin/EDTA (TE) solution to the cells and incubate at room temperature or 37°C for 5-10min, or until the cells attach.
- 4) Examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.
- 5) Resuspend cells in 2% FBS in PBS and transfer to a 15ml tube.
- 6) Centrifuge cells at 200×g for 4min.
- 7) Aspirate supernatant and gently resuspend cell pellet in Freezing medium (approx. 1×10⁶ cells/ml).
- 8) Transfer cell suspension into cryovial(s) and place them into a freezing container ("Mr. Frosty" or similar).
- 9) Place the freezing container at -70 to -80°C for 16-24h.
- 10) Transfer cryovials to liquid nitrogen vapor for long-term-storage.

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