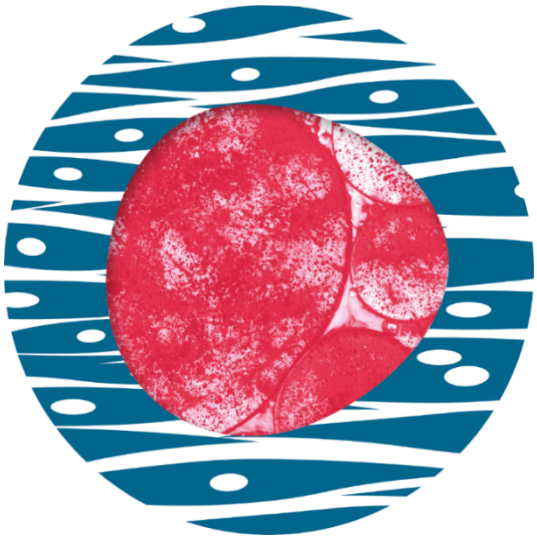
CI-muHepa

Immortalized murine hepatocytes

Product Sheet

→ At a glance		
BSL *Level 1*	***Coating*** *Collagen Solution (INS-SU-1017)*	***Growth*** *Adherent*
Storage *<2d: -80°C* *>2d: liquid Nitrogen*	***Medium*** *Lonza HCM plus 5%FBS (Lonza #CC-3198)*	***Cat.-No.*** *INS-CI-1012*



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→ Intended Use and Licensing

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→ Background Information

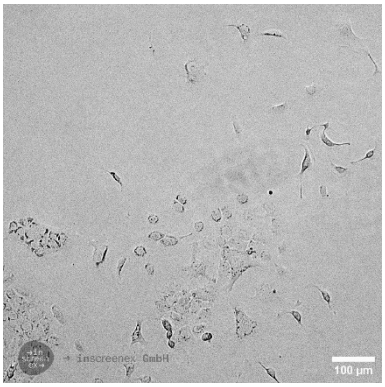
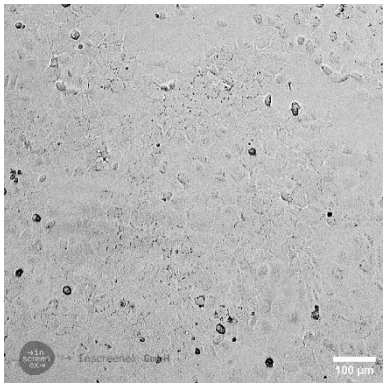
CI-muHepa is an immortalized murine hepatocyte cell line

Catalog number: *INS-CI-1012*
Biosafety level (BSL): *Level 1*
Organism: *Mus musculus (murine)*
Tissue: *liver*
Growth properties: *adherent*

CI-muHepa are immortalized murine hepatocytes. Hepatocytes, the major parenchymal cells in the liver, play pivotal roles in metabolism, detoxification, and protein synthesis.

→ Morphology

adherent; grows in monolayer

Low confluency	***High confluency***
	

→ Cell Characterization and Assays

→ Marker Expression

The following cell-type specific markers were detected in CI-muHepa:
Albumin, Hnf4alpha, G6pc

→ Established Assays

CI-muHepa were used in the following assays:

Cyp activity

Glycogen storage

Growth in 3D Culture

→ Quality Control

Basic information on quality control can be found below. For more details, request a Certificate of Analysis (CoA) by emailing **info@inscreenex.com** *and stating your Lot number.*

Cell number: *>0.5Mio viable cells*
Viability: *>75% post-thaw viability*
Sterility: *no contamination detected*
Mycoplasma: *no contamination detected*
Human pathogens: *-*
STR: *Not available*

→ Related Products

Products that are related to the CI-muHepa cell line and are either required for a successful cell culture or recommended.

Required	***Recommended***
Medium: *Lonza HCM plus 5%FBS (Lonza #CC-3198)* ***Coating solution:*** *Collagen Solution (INS-SU-1017)*	***Cryopreservation:*** *Cell Freezing Medium (INS-SU-1027)* ***Related cell lines:***

→ Upon Arrival

Cells are routinely shipped on dry ice. Check all containers for leakage and breakage. Check if cells arrived frozen.

If, immediately upon arrival:

- *the vial appears damaged,*
- *the dry ice level in the shipping container appears low,*
- *the cells appear thawed, or*
- *you have any other concerns regarding the quality of the cells,*

do the following:

1) *take photos of the vial and/or the shipping container,*
2) *contact us by email or telephone (see* **General Inquiries** *on page 2).*

If everything looks good, either seed the cryopreserved cells immediately, or store them:

- *at -80°C for periods of up to 2 days, or*
- *below -130°C in liquid nitrogen vapor, for long term storage.*

→ Coating Protocol

Our *Collagen Solution is a sterile, ready-to-use solution ideal for coating cell culture surfaces.*

Required materials	***Protocol***
– *Cell culture plastic (flasks, dishes, plates* – *Sterile PBS* – *Collagen Solution (INS-SU-1017)*	*1) Cover the cell culture vessel with Collagen Solution (see table to the left for recommended volumes). Rock back and forth to cover entire surface if necessary.* *2) Incubate for at least 2h at 37°C in the incubator or overnight at 2-8°C.* *3) Aspirate Collagen Solution.* *4) Wash once with at least 4 volumes of sterile PBS.* *5) Aspirate PBS.* *6) Use immediately or store coated cell culture plastic according to instructions provided to the left.*
Storage	
– *Store Collagen Solution at 2-8°C, if not indicated otherwise on the product label.* – *Store coated cell culture plastic sealed at 2-8°C for up to 7 days.*	

Recommended volumes	

Flask/Plate	Coating Volume
T75	3ml
T25	1ml
6-well	0.7ml
12-well	0.25ml
24-well	0.1ml
48-well	75µl
96-well	50µl

→ Medium Storage and Preparation

Lonza HCM plus 5%FBS) Bullet Kit (#CC-3198) consists of:

- HCM™ SingleQuots™ Kit (CC-4182)
- HBM™ Hepatocyte Basal Medium, 500 mL (CC-3199)

The components need to be combined according to the manufactures instructions to obtain the complete, ready-to-use medium. Please note that FBS needs to be added to a final concentration of 5%.

→ Cell culture antibiotics: The medium does not contain prophylactic antibiotics. If you wish to use antibiotics, any standard, cell culture grade antibiotics can be added to the medium.

Required materials	**Protocol**
– Lonza HCM Bullet Kit (#CC-3198) – Cell culture grade FBS/FCS	1) Thaw Supplements at ambient temperature. 2) Combine Supplements and FBS to 500ml Basal Medium to obtain the complete Medium.
Storage	
– Store **Supplements** at -20°C, if not indicated otherwise on the product label.	To prepare smaller volumes of complete Medium, aliquot Supplements and store at -20°C. For

– *Store* **Basal Medium** *at 2-8°C, if not indicated otherwise on the product label.* – *Store* **complete Medium** *at 2-8°C. Complete Medium is stable for at least 1 month at 2-8°C.*	*example, aliquot 5×6ml and then add 6ml Supplements to 100ml Basal Medium.*

→ Thaw Cryopreserved Cells

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

Required materials	***Protocol***
– *Coated cell culture vessel (see section* **→ Coating** Protocol *on page 5)* – *Complete Medium, pre-warmed to 37°C (see section* **→ Medium Storage and** Preparation *on page 6)* – *15ml tube with a conical bottom suitable for centrifugation (e.g. "Falcon tube")*	*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 by spraying with 70% Ethanol.* *3) Transfer thawed cell suspension to the 15ml tube containing 4ml medium. Avoid excessively pipetting up and down.* *4) Centrifuge cells at 300×g for 5min.* *5) Aspirate supernatant.* *6) Gently resuspend the cell pellet in complete Medium. Use a volume appropriate for the cell culture vessel.* *7) Transfer cells in coated cell culture vessel and place in the incubator (37°C, 5% CO2).* *8) Change the medium after 2 days.*

→ Freeze Cells for Cryopreservation

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

Required materials	***Protocol***
– *Cell Freezing medium (INS-SU-1027)* – *PBS* – *Trypsin/EDTA solution (TE)* – *2% FBS in PBS* – *15ml tube* – *Cryovial(s)* – *Freezing container ("Mr. Frosty" or similar)* – *15ml tube with a conical bottom suitable for centrifugation (e.g. "Falcon tube")*	*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 2% FBS in PBS and transfer to a 15ml conical bottom tube.* *6) Centrifuge cells at 300×g for 5min.* *7) Aspirate supernatant and gently resuspend cell pellet in Freezing medium (approx. 1Mio. 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-80°C for 16-24h.* *10) Transfer cryovials to liquid nitrogen vapor for long-term-storage. Add 4ml pre-warmed medium to a 15ml tube.*

→ Routine Cell Culture

Work in a sterile environment and follow Good Cell and Tissue Culture Practice.

Temperature: *37°C*
Environment: *5% CO_2 (v/v), humidified atmosphere*
Split ratio: *1:2 for initial split after thawing, 1:5 to 1:10 for routine culture*
Confluence: *split at 70-90% confluence*
Medium change: *every 2-3 days*

Required materials	***Protocol***
– *Coated cell culture vessel (see section* → **Coating** Protocol *on page 5)* – *Complete Medium, pre-warmed to 37°C (see section* → **Medium Storage and** Preparation *on page 6)* – *PBS* – *Trypsin/EDTA solution (TE)*	*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) Place into incubator.*
Recommended volumes	

Flask or Plate	***Medium or PBS***	***TE solution***
T75	*8-10ml*	*3ml*
T25	*4-5ml*	*1ml*
6-well	*1.5-3ml*	*0.7ml*
12-well	*1-2ml*	*0.25ml*
24-well	*0.5-1ml*	*0.1ml*
48-well	*0.2-0.4ml*	*75µl*
96-well	*0.1-0.2ml*	*50µl*

→ References

Lipps, Christoph, et al. 'Expansion of Functional Personalized Cells with Specific Transgene Combinations'. Nature Communications, vol. 9, no. 1, Mar. 2018, p. 994, https://doi.org10.1038/s41467-018-03408-4.

Wahlicht, Tom et al. “Controlled Functional Zonation of Hepatocytes In Vitro by Engineering of Wnt Signaling.” ACS synthetic biology vol. 9,7 (2020): 1638-1649. doi:10.1021/acssynbio.9b00435