Lonza Walkersville, Inc.
Walkersville, MD 21793-0127 USA
U.S. Scientific Support: 800 521 0390
scientific.support@lonza.com
EU/ROW Scientific Support: +49 221 99199 400
scientific.support.eu@lonza.com
www.bioscience.lonza.com

Hepatocyte and Non-Parenchymal Cell (NPC) 2D High-Throughput Co-Culture

Instructions for use

Safety Statements

These products are not for use in GMP manufacturing, nor human or animal *in vivo* use, including use as a diluent or as an excipient, or for diagnostic use.

These products are for research use *only.*

WARNING: LONZA PRIMARY CELLS CONTAIN HUMAN SOURCE MATERIAL; TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-I, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV-1, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human-sourced products should be handled at the biological safety level 2 to minimize exposure to potentially infectious products, as recommended in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th edition. If you require further information, please contact your site safety officer or Scientific Support.

Preparation of reagents

All work should be performed in a laminar flow hood. Decontaminate the external surfaces of all supplement vials and the medium bottles with ≥70% ethanol or isopropanol.

1. **Hepatocyte Media:**
   a. **Hepatocyte Plating Medium:** Prepare as per manufacturer instructions, adding 1 mL of the plating supplement per 50 mL of Hepatocyte Plating Base Medium.
   b. **Hepatocyte Culture Medium (HCM™ Completed Medium):** Prepare as per manufacturer instructions, adding all SingleQuots® supplements to the Hepatocyte Base Medium (HBM™).
      i. **OPTIONAL:** If utilizing this co-culture for cell culture assays that model or trigger an inflammatory response, you may consider leaving out the hydrocortisone SingleQuots® supplement as it can suppress inflammatory cytokine production by Kupffer Cells (KCs). However, this effect is not large enough to impact the biologic relevance of the model, so this choice is discretionary.
2. **Basement Membrane Matrix Working Solution**
   a. Add the basement membrane matrix (Corning® Matrigel®) Stock Solution to **HCM™ Completed Medium** to make a final concentration of 0.3 mg/mL
      i. Basement Membrane Matrix Stock Solution concentrations varies by batch, so scale ratio of Stock Solution to HCM™ accordingly.

Hepatocyte Preparation

NOTE: All work is to be performed in a laminar flow hood.

NOTE: Cells will be plated in 96-well collagen-coated plates. Prepare prior to thawing hepatocytes or purchase pre-prepared collagen-coated plates.

1. Pre-warm a 50 mL conical tube of **Hepatocyte Thawing Medium**.
2. Thaw Hepatocytes at 37°C for 90–120 seconds, until a sliver of ice remains.
3. Transfer cells to the 50 mL conical tube of pre-warmed Hepatocyte Thawing Medium.
4. Suspend the cells by carefully rocking the 50 mL tube by hand for a few seconds. DO NOT VORTEX.
5. Centrifuge the cells at 100xg for 8 minutes at room temperature. Aspirate the supernatant.

6. Add 3–4 mL pre-warmed **Hepatocyte Plating Medium** to the 50 mL tube. Gently invert a few times to suspend the cells. DO NOT VORTEX.
7. Count cells using trypan blue and a hemocytometer using a 1:10 dilution with trypan blue.
    a. Add 400 µL Hepatocyte Plating Medium to a separate microcentrifuge tube
    b. Add 50 µL 0.4% trypan blue to the new microcentrifuge tube
    c. Transfer a 50 µL aliquot of the cell suspension to the new tube
8. After counting, dilute the cell suspension in each tube to 500,000 cells/mL with Hepatocyte Plating Medium. Invert gently to mix. DO NOT VORTEX.
9. Plate 100 µL of the hepatocyte cell suspension in each well of a collagen-coated 96-well plate.
    a. Plate as many wells as required, and then fill all remaining wells with 100 µL PBS. Plate hepatocytes from the center of the plate outwards so that any unused wells to be filled with PBS are towards the outside of the plate.
10. Place plate in the incubator for 1 hour at 37°C and 5% CO_2. Do not disturb.

NPC Preparation and Co-Culture Establishment

NOTE: All work is to be performed in a laminar flow hood.

NOTE: Plate NPCs at 1 hour after Hepatocyte plating.

1. Thaw NPCs at 37°C for 90–120 seconds until only a sliver of ice remains.
    a. Kupffer Cells (KCs)
    b. Stellate Cells (SCs)
    c. Liver-derived Endothelial Cells (LECs)
2. Prepare 3 (three) 15 mL conical centrifuge tubes containing 10 mL cold Hepatocyte Plating Medium, one for each NPC type used.
3. Add each type of NPC to its appropriate conical tube.
4. Centrifuge cells at 300xg for 10 minutes at 4°C.
5. Remove supernatant, then resuspend cells in each conical tube as follows:
    a. KCs and SCs: 500 µL cold Hepatocyte Plating Medium
    b. LECs: 1 mL cold Hepatocyte Plating Medium
6. Count cells using trypan blue and a hemocytometer using a 1:10 dilution with trypan blue.
    a. Example: Add 200 µL Hepatocyte Plating Medium to a separate microcentrifuge tube. Add 25 µL of the cell suspension to this tube followed by 25 µL trypan blue.
7. Calculate the volume of each NPC cell suspension needed such that, when combined together, the total ratio of 1 hepatocyte to each NPC will equal:
    a. Kupffer Cells: 0.25
    b. Stellate Cells: 0.13
    c. Liver-derived Endothelial Cells: 0.29
    d. Each well of the 96-well plate already contains 50,000 hepatocytes. You should therefore add to each well:
        i. 12,500 KCs
        ii. 6,500 SCs
        iii. 14,500 LECs
    e. EXAMPLE: If plating a total of 70 co-culture wells, you would need:
        i. 8.75×10^5 KCs
        ii. 4.55×10^5 SCs
        iii. 1.015×10^6 LECs
        iv. After cell counting, combine the above number of each cell type into a single 15 mL conical centrifuge tube
        v. Dilute to 7 mL with Hepatocyte Plating Medium. Gently invert to mix. DO NOT VORTEX.
    f. Use the above examples to scale your volumes and # of cells needed appropriately based on the total # of co-culture wells you wish to plate.
8. Take the hepatocyte plate from the incubator and aspirate supernatant from each co-culture well.
9. Add 100 µL of the combined NPC cell suspension to each co-culture well.
10. Incubate cells at 37°C and 5% CO_2 for 4–5 hours.
11. After 4–5 hours of incubation, aspirate plating medium with nonattached cells.
12. Feed cells in each well 100 µL of pre-chilled **Basement Membrane Matrix Working Solution.**
13. Place cells back in the incubator and incubate overnight.
14. Change media the following day, replacing the Basement Membrane Matrix Working Solution with **HCM™ Completed Medium**.
15. Change medium in each well with fresh HCM™ Completed Medium every day thereafter until day 5.

NOTE: This protocol was tested out to 5 days of co-culture, at which point the hepatocytes and NPCs were still functional and healthy.

NOTE: This co-culture model can be used for a variety of high-throughput applications, such as drug-induced liver functionality studies, drug-drug interaction (DDI), and other ADME-toxicology applications.

Ordering Information

Catalog No.	Description	Size
HUCPI	Cryopreserved Human Hepatocytes	≥ 5 million cells
HLKC-500K	Cryopreserved Human Kupffer Cells	≥ 0.5 million cells
HUCLS-200K	Cryopreserved Human Stellate Cells	≥ 0.2 million cells
HLECP1	Cryopreserved Human Liver-derived Endothelial Cells (LECs)	≥ 1 million cells
MCHT50	Hepatocyte Thawing Media	50 mL
MP250	Hepatocyte Plating Medium with Supplement	250 mL + supplement
CC-3199	HBM Basal Medium	500 mL
CC-4182	HCM™ SingleQuots® Supplements	1 kit
CC-3198	HCM™ Hepatocyte Culture Medium BulletKit®	1 kit

Corning® Matrigel® Basement Membrane Matrix, LDEV-free, 10mL (part no. 354234) mentioned is a product of Corning®.

 CD-OP030 09/24