

3D Spheroid Primary Human Hepatocyte and Non-Parenchymal Cell (NPC) Co-Culture

Instructions for use

Safety Statements

These products are not for human or animal *in vivo* or diagnostic use, including use as a diluent or as an excipient.

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-1, 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. Spheroid Formation Medium

Consists of Lonza Hepatocyte Culture Medium (HCM – complete medium, see below) with 20% FBS and 25 mM HEPES. For example, combine 77.5 mL HCM with 20 mL FBS and 2.5 mL 1M HEPES.

2. Hepatocyte Culture Medium (HCM Complete Medium)

Transfer contents of HCM SingleQuots® Kit (Lonza part no. CC-4182) to HBM Basal Medium (Lonza part no. CC-3199) with a pipette and rinse each vial with medium. Store at 4°C for up to 1 month.

Co-Culture Preparation

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

1. Warm Lonza **Hepatocyte Thawing Medium** (Lonza part no. MCHT50) in a 37°C water bath.
2. Prepare 3 separate 15 mL conical tubes with **cold** 10 mL **Spheroid Formation Medium** and label KC (Kupffer Cells), SC (Stellate Cells), and LEC (Liver-derived Endothelial Cells), respectively.
3. Thaw Hepatocytes (Lonza cat. no. HUCPI or HUCPG) and Non-Parenchymal Cells (NPCs; i.e., KC, SC and LEC) in the 37°C water bath until only a sliver of ice remains (about 90 – 120 seconds). **DO NOT SUBMERGE.**
4. Disinfect vial with ethanol or isopropanol and transfer to the BSC.
5. Carefully pour or pipette (with a wide-bore tip) **hepatocytes** into the 50 mL conical tube of **Hepatocyte Thawing Medium**.
 - a. Pipette 1 mL of Hepatocyte Thawing Medium into the original vial to rinse. Pour or pipette this back into the 50 mL conical tube of Hepatocyte Thawing Medium.
 - b. Suspend cells by carefully rocking the 50 mL tube by hand for a few seconds. **DO NOT VORTEX.**

6. Carefully pour or pipette (with a wide-bore tip) each **NPC** into its respective 15 mL tube (KC, SC, and LEC) prepared in Step 2.
7. Centrifuge cells:
 - a. Hepatocytes: 100xg for 8 minutes at room temperature.
 - b. NPCs: 300xg for 10 minutes at 4°C.
8. Remove the supernatant from all tubes.
9. Resuspend the cells in:
 - a. Hepatocytes: 2 mL of Spheroid Formation Medium; rock gently to mix (DO NOT PIPETTE MIX).
 - b. NPCs: 1 mL of Spheroid Formation Medium; pipette to mix.
10. Count cells using Trypan Blue and a hemocytometer to determine viability and yield using the following guidance:
 - a. Hepatocytes: use a 1:20 dilution.
 - i. E.g., add 50 µL of 0.4% Trypan Blue to a clean microcentrifuge tube. Add 425 µL Spheroid Formation Medium and 25 µL of Hepatocyte cell suspension.
 - ii. Other volumes may be used as long as Trypan Blue represents no more than 10% of total volume.
 - b. NPCs:
 - i. SC and KC: use a 1:2 or 1:5 dilution
 1. E.g., add 50 µL of 0.4% Trypan Blue, and 50 µL of cell suspension to a clean microcentrifuge tube for a 1:2 dilution.
 - ii. LEC: use a 1:10 dilution
 1. E.g., add 90 µL of 0.4% Trypan blue and 10 µL of cell suspension to a clean microcentrifuge tube for a 1:10 dilution.
11. Adjust cells to final concentrations as follows:
 - a. Hepatocytes: 1 x 10⁶ cells/mL.
 - b. NPCs: 5 x 10⁵ cells/mL.
12. Combine all cells into a single 15 mL tube in the following volumes:
 - a. 195 µL Hepatocytes.
 - b. 49 µL SC.
 - c. 59 µL KC.
 - d. 87 µL LEC.
 - e. Gives a final ratio of 1 Hepatocyte : 0.25 SC : 0.30 KC : 0.45 LEC.
13. Dilute the co-culture cell suspension to 13 mL with Spheroid Formation Medium.
 - a. Provides enough volume to seed ~130-wells using a 96-well plate format at 22,500 cells/mL.
14. Plate cells in a 96-well ultra-low attachment plate using 100 µL of cell suspension per well.
15. Each well will have approximately 1,500 hepatocytes, 188 SC, 225 KC, and 337 LEC.
16. Fill an outer ring of wells around the cells with Spheroid Formation Medium or 1X PBS to help control evaporation during culture.
17. Place plate into a humidified incubator at 37°C, 5% CO₂. Leave undisturbed (no medium changes, etc.) until at least day 5.
18. Perform 50% medium changes on days 5 – 7, carefully pipetting out medium from the side of the wells (place pipette tips at 45 degree angle) so as not to pipette out the spheroids, and adding fresh, pre-warmed **Hepatocyte Culture Medium (HCM Complete Medium)**.
19. After day 7, perform a 50% medium change using fresh, pre-warmed HCM as in Step 18 every 2–3 days through day 21.

Ordering Information

| Catalog No. | Description | Size |
|-------------|---|-------------------|
| HUCPG | Cryopreserved Primary Human Hepatocytes, Plateable | ≥ 5 million cells |
| HUCPI | Cryopreserved Primary Human Hepatocytes, Plateable, DDI Qualified | ≥ 5 million cells |
| HUCLS-200K | Cryopreserved Human Stellate Cells (SC) | ≥ 200,000 cells |
| HLKC-500K | Cryopreserved Human Kupffer Cells (KC) | ≥ 500,000 cells |
| HLECP1 | Cryopreserved Human Liver-derived Endothelial Cells (LEC) | ≥ 1 million cells |
| MCHT50 | Human Hepatocyte Thawing Media | 50 mL |
| CC-3199 | HBM Basal Medium | 500 mL |
| CC-4182 | HCM SingleQuots® Supplements | 1 kit |
| CC-3198 | HCM Hepatocyte Culture Medium BulletKit® | 1 kit |
| BEBP17-737E | 1M HEPES Buffer | 100 mL |

FBS (VWR 97068-085) mentioned is a product of Avantor.

GIBCO® Trypan Blue (15250061) mentioned is a product of Thermo Fisher Scientific.

96-well ultra-low attachment plates (Corning CLS7007) mentioned are a product of Corning®.

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