

## 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. Plating 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 **Plating 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 Plating Medium; rock gently to mix (DO NOT PIPETTE MIX).
  - b. NPCs: 1 mL of Plating 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 Plating 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 10<sup>6</sup> cells/mL.
  - b. NPCs: 5 x 10<sup>5</sup> 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 Plating 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 Plating Medium or 1X PBS to help control evaporation during culture.
17. Place plate into a humidified incubator at 37°C, 5% CO<sub>2</sub>. 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®.

For research use only. Not for use in diagnostic procedures.

All trademarks belong to Lonza, and are registered in the USA, EU and/or CH or belong to third-party owners and are used only for informational purposes. All third-party copyrights have been reproduced with permission from their owners. The information contained herein is believed to be correct and corresponds to the latest state of scientific and technical knowledge. However, no warranty is made, either expressed or implied, regarding its accuracy or the results to be obtained from the use of such information and no warranty is expressed or implied concerning the use of these products. The buyer assumes all risks of use and/or handling. Any user must make his own determination and satisfy himself that the products supplied by Lonza Group Ltd or its affiliates and the information and recommendations given by Lonza Group Ltd or its affiliates are (i) suitable for intended process or purpose, (ii) in compliance with all applicable laws, including all environmental, health and safety regulations, and (iii) will not infringe any third party's intellectual property rights. The user bears the sole responsibility for determining the existence of any such third-party rights, as well as obtaining any necessary licenses and approvals. For more details:

<http://www.lonza.com/legal>

© 2024 Lonza. All rights reserved.

CD-OP025 02/24.