

Long-Term Culture of Primary Human Hepatocyte 3D Spheroids and Cytotoxicity Assay

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 34.875 mL HCM with 9 mL FBS and 1.125 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.

Hepatocyte Preparation

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

1. Warm Lonza **Hepatocyte Thawing Medium** (Lonza part no. MCHT50) and Spheroid Formation Medium in a 37°C water bath.
2. Remove Verified for Spheroids Human Hepatocytes (Lonza cat. no. HUCPI or HUCPG) and thaw in the 37°C water bath until only a sliver of ice remains (about 90–120 seconds). **DO NOT SUBMERGE.**
 - a. NOTE: Lonza routinely screens plateable hepatocyte lots for spheroid formation potential. To learn which lots are characterized for spheroid formation, contact Scientific Support at scientific.support@lonza.com or scientific.support.eu@lonza.com
3. Disinfect vial with ethanol or isopropanol and transfer to the BSC.
4. Carefully pour or pipette (with a wide-bore tip) hepatocytes into the 50 mL conical tube of **Hepatocyte Thawing Medium**.
5. 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.

6. Suspend the cells by carefully rocking the 50 mL conical tube by hand for a few seconds. **DO NOT VORTEX.**
7. Centrifuge hepatocytes at room temperature at 100xg for 8 minutes.
8. Remove the supernatant.
9. Resuspend the cells in 3 mL pre-warmed **Spheroid Formation Medium.**
10. Count cells using Trypan Blue and a hemocytometer to determine viability and yield using the following guidance:
 - a. Add 50 µL of 0.4% Trypan Blue to a clean microcentrifuge tube. Add 350 µL Spheroid Formation Medium and 100 µL of Hepatocyte cell suspension
 - i. Represents a 1:5 dilution
 - ii. Other volumes may be used as long as Trypan Blue represents no more than 10% of total volume.
11. Adjust cells to a final concentration of 1.5 x 10⁴ cells/mL in Spheroid Formation Medium.
12. Plate cells in a 96-well ultra-low attachment plate using 100 µL of cell suspension per well for a total of 1,500 cells/well.
 - a. Fill an outer ring of wells around the cells with Spheroid Formation Medium or 1X PBS to help control evaporation during spheroid formation
13. Place plate into a humidified incubator at 37°C, 5% CO₂. Leave undisturbed (no medium changes, etc.) until at least day 5.
14. Perform 50% medium changes on days 5 and 6, 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).**
15. On day 7, prepare **2X Drug Dosing Medium** by dissolving target drug in **Hepatocyte Culture Medium** at an appropriate concentration and titrating down the plate
 - a. Example: Using acetaminophen, create a stock solution in HCM of 2X your desired highest dose (e.g., 50 mM for a desired highest dose of 25 mM). Titrate the 2X Drug Dosing Medium in HCM to produce 8 concentrations ranging from 0–50 mM.
16. Perform 50% medium changes with **2X Drug Dosing Medium** on day 7.
 - a. Note: this results in the final desired drug concentration, e.g., 25 mM
17. Every 2–3 days thereafter up to 21 days (for a total of 28 days in culture), perform 50% media changes with **1X Drug Dosing Medium.**
 - a. Example: Using acetaminophen, create a stock solution in HCM of your desired highest dose (e.g., 25 mM). Titrate the 1X Drug Dosing Medium in HCM to produce 8 concentrations ranging from 0–25 mM.
18. A variety of assessments can be utilized to assess spheroid health and functionality and generate EC₅₀ values for the drug being tested.
 - a. Example: Set up plates to be harvested at appropriate time points (e.g., 24–72 hours post-dosing for acute toxicity studies or 7 to 14 days post-dosing for chronic toxicity studies). Using Lonza's ViaLight® Plus BioAssay Kit, test cytotoxicity at each time point based on total cellular ATP to generate dose response curves and EC₅₀ values.
 - b. Example: Using an Incucyte® Live Cell Imaging System for live-cell imaging, Cytotox Green Dye can be added to the drug dosing medium according to manufacturer instructions and cells can be monitored continuously for cytotoxicity over several time points in culture.

Ordering Information

Catalog No.	Description	Size
HUCPG	Cryopreserved Primary Human Hepatocytes, Plateable (Verified for Spheroids)*	≥ 5 million cells
HUCPI	Cryopreserved Primary Human Hepatocytes, Plateable, DDI Qualified. (Verified for Spheroids)*	≥ 5 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
LT07-321	Lonza ViaLight®	10,000 test kit
LT07-121	Plus BioAssay	1,000 test kit
LT07-221	Kit	500 test kit

* **Ask for lots verified for Spheroids:** Lonza routinely screens plateable hepatocyte lots for spheroid formation potential. To learn which lots are characterized for spheroid formation, contact Technical Support at scientific.support@lonza.com or scientific.support.eu@lonza.com

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®.

Acetaminophen (Sigma A5000-100G) mentioned is a product of Sigma Aldrich.

Incucyte® Live Cell Analysis System mentioned is a product of Sartorius.

Incucyte® Cytotox Green Dye (Essenbio 4633) mentioned is a product of Sartorius.

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