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Hepatocyte and Non-Parenchymal Cell (NPC) 2D 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. **DMEM Plating Medium**
   a. 45 mL DMEM (high glucose, no glutamine or phenol red)
   b. 500 µL 100X ITS
   c. 1 mL GLutaMAX™
   d. 50 µL 1000X Gentamicin/Amphotericin
   e. 250 µL 200X Hydrocortisone (to make a final concentration of 1 µM)
2. **DMEM Maintenance Medium**
   a. 50 mL DMEM (high glucose, no glutamine or phenol red)
   b. 500 µL 100X MEM non-essential amino acids (NEAA)
   c. 500 µL 100X ITS
   d. 1mL GlutaMAX™
   e. 50 µL 1000x Gentamicin/Amphotericin
3. **Matrigel® Working Solution**
   a. Add 208 µL of Matrigel Stock Solution (10 mL) to 6.792 mL DMEM Maintenance Medium to make a final concentration of 0.3 mg/mL. Scale up or down as needed.

Hepatocyte Preparation

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

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 3mL pre-warmed DMEM 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. Example: Add 200 µL DMEM plating media to a separate microcentrifuge tube. Add 25 µL of the cell suspension to this tube followed by 25 µL Trypan Blue.

8. After counting, dilute the cell suspension to 1 x 10^6 cells/mL using DMEM Plating Medium. Invert gently to mix. DO NOT VORTEX.
9. Plate 500 µL of Hepatocytes into the appropriate wells of a 24-well collagen-coated plate.
10. Place plate in the incubator for 1 hour at 37°C and 5% CO_2. Gently rock the plate every 15 minutes.

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
   b. Stellate Cells
   c. Liver-derived Endothelial Cells
2. Prepare 3 conical tubes containing 10 mL cold DMEM 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 in 1 mL of cold DMEM Plating Medium.
6. Count cells using Trypan Blue and a hemocytometer using a 1:10 dilution with Trypan Blue.
   a. Example: Add 200 µL plating media to a separate microcentrifuge tube. Add 25 µL of the cell suspension to this tube followed by 25 µL Trypan Blue.
7. Calculate the amount of DMEM Plating Medium needed to dilute each cell type such that the ratio of each NPC to hepatocytes will be as follows once plated:
   a. Kupffer Cells: 0.25 – 0.29
   b. Stellate Cells: 0.13 – 0.15
   c. Liver-derived Endothelial Cells: 0.25 – 0.29
   d. EXAMPLE: Add the following to each well in a 24-well plate:
      i. 500,000 Hepatocytes
      ii. 125,000 – 145,000 Kupffer Cells
      iii. 65,000 – 75,000 Stellate Cells
      iv. 125,000 – 145,000 Liver-derived Endothelial Cells
   e. NOTE: Final volume in each well should be 500 µL total after plating all NPCs plus hepatocytes. You can plate smaller volumes of each cell type to get ratios correct and then add media to bring volume to 500 µL.
8. Take the hepatocyte plate from the incubator and aspirate supernatant from each well.
9. Add NPC at the calculated volumes above to each well.
10. IF NEEDED: Bring total volume of each well to 500 µL using DMEM Plating Medium (depends on total volume across all NPCs added to each well to achieve desired ratios with hepatocytes).
11. Incubate cells at 37°C and 5% CO_2 for 1 hour, gently rocking every 15 minutes to evenly disperse cells.
12. After incubation, aspirate plating medium with nonattached cells.
13. Feed cells in each well 500 µL of pre-warmed DMEM Maintenance Medium.
14. Place cells back in the incubator and incubate for another 4-5 hours.

Matrigel® Overlay

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

1. At 4-5 hours after co-culture establishment, remove the plate from the incubator and aspirate the media.
2. Add 500 µL of Matrigel® Working Solution to each well.
3. Place the plate back in the incubator at 37°C and 5% CO_2.
4. Change media to DMEM Maintenance Medium the next day and every day thereafter.
5. NOTE: This protocol was tested out to 7 days of co-culture, at which point the hepatocytes and NPCs were still functional and healthy.
6. This co-culture model can be used for a variety of applications, such as drug-induced liver functionality studies.

Ordering Information

Catalog No.	Description	Size
HUCPI	Cryopreserved Human Hepatocytes	≥ 5 million cells
HLKC-500K	Cryopreserved Human Kupffer Cells	≥ 500 thousand cells
HUCLS-200K	Cryopreserved Human Stellate Cells	≥ 200 thousand cells
HLECP1	Cryopreserved Human Liver-derived Endothelial Cells (LECs)	≥ 1 million cells
MCHT50	Hepatocyte Thawing Media	50 mL
CC-4083	1000X Gentamicin/Amphotericin	5 mL

Avantor®, Seradigm Select Grade Fetal Bovine Serum FBS (VWR 97068-085) mentioned is a product of Avantor®.

GIBCO™ DMEM (31053028) mentioned is a product of Thermo Fisher Scientific.

GIBCO™ 100X MEM Non-Essential Amino Acids (11140050) mentioned is a product of Thermo Fisher Scientific.

GIBCO™ GlutaMAX™ supplement (35050079) mentioned is a product of Thermo Fisher Scientific.

Corning® ITS (Insulin-transferrin_Selenium),100X (25-800-CR) mentioned is a product of Corning®.

200X Hydrocortisone 96 µg/mL (07925) mentioned is a product of StemCell Technologies™.

GIBCO™ Trypan Blue 0.4% (ThermoFisher 15250061) mentioned is a product of Gibco™.

Corning® BioCoat® 24-well collagen coated plates (354408) mentioned is a product of Corning®.

Corning® Matrigel® Basement Membrane Matrix, LDEV-free, 10mL) (354234) mentioned is a product of Corning®