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Human Liver Endothelial Cells (HLEC)

Instructions for Use

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For answers to frequently asked questions regarding these products, please visit our FAQ Database:

www.lonza.com/faq

For references citing the use of these products, please visit our Citation Database:

www.lonza.com/citations

I. Required Materials

1. HLEC (Lonza catalog number HLECP2)
2. EGM-2™ BulletKit™ Medium (Lonza catalog number CC-3162)
3. PBS/Wash Buffer (Lonza catalog number 17-516F or equivalent)
4. 0.25%Trypsin/EDTA (Lonza catalog number 17-161E)
5. Corning™ BioCoat™ Collagen I, Rat Tail Culture Dish (Corning catalog number 354450)

II. Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells, remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact Scientific Support at the email address address provided in the header to this page.
3. Lonza's BulletKit™ Medium Instructions: upon arrival, store Basal Medium at 2°-8°C and SingleQuots™ Kit at -20°C in a freezer that is not self-defrosting. If thawed upon arrival, growth factors can be stored at 2° -8°C and added to the basal medium within 72 hours of receipt. After the SingleQuots™ Kit supplements are added to basal medium use within one month. Do not re-freeze.

NOTE: Using media or reagents other than the recommended ones will void the cell warranty. Please contact Scientific Support if you need help selecting media and/or reagents.

III. Preparation of BulletKit™ Medium

1. Decontaminate the external surfaces of all supplement vials and the medium bottle with ethanol or isopropanol.
2. Aseptically open each supplement vial and add the entire contents to the basal medium with a pipette.
3. Rinse each cryovial with the medium. It may not be possible to recover the entire volume listed for each cryovial. Small losses, even up to 10%, should not affect the cell growth characteristics of the supplemented medium.
4. Transfer the label provided with each kit to the basal medium bottle being supplemented. Use

it to record the date and amount of each supplement added. We recommend that you place the completed label over the basal medium label (avoid covering the basal medium lot # and expiration date) to avoid confusion or possible double supplementation. **After adding supplements, the complete medium has a shelf life of one month. Do not freeze medium.**

5. Record the new expiration date on the label based on the shelf life.

NOTE: If there is concern that the sterility was compromised during the supplementation process, newly prepared medium may be re-filtered with a 0.2-micron filter to assure sterility. Routine filtration/re-filtration is not recommended.

IV. Thawing of Cells / Initiation of Culture Process

1. Clean the exterior of the complete medium bottle with 70% ethanol before use. Transfer a 5 mL aliquot of the complete medium to a sterile 15 mL conical tube and place in a 37°C water bath to pre-warm.
2. Transfer the pre-warmed 5 mL aliquot of medium to a clean sterile 15 mL conical tube, place cap on conical tube and maintain at room temp.
3. Wipe cryovial of cells with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure and then retighten.

NOTE: Thawing the cells for longer than 2 minutes results in less than optimal results.

4. Quickly thaw the cryovial in a 37°C water bath being careful not to submerge the entire vial. Watch the cryovial closely; when the last sliver of ice melts, remove the vial from the water bath.
5. Transfer the entire contents of the cryovial into the conical tube prepared in step 3. Gently pipette the cell suspension up and down.
6. Centrifuge the cell suspension at 200 x g for 5 minutes at room temperature.
7. Gently aspirate the supernatant. Then re-suspend the cells in 8.0 mL of complete medium.
8. Perform a cell count to determine the total number of viable cells.

NOTE: The plating recommendations below are for a 100 mm dish. Adjust all volumes accordingly for other size culture vessels.

9. Pre-fill the culture vessels with 5.0 mL of complete medium.
10. Dispense the cells at a seeding density of 3000 cells/cm^2 or 230,000 cells per 100 mm dish.
11. Immediately swirl the plate gently to distribute the cells.
12. Bring the total volume in each culture vessel to 10.0 mL.
13. Place the culture vessels in a 37°C incubator at 5% CO_2.

V. Maintenance

1. Change the medium every 3-4 days with 10.0 mL of freshly warmed complete medium per 100 mm dish. Adjust all volumes accordingly for other size culture vessels.

NOTE: Only warm aliquots of medium. Repeatedly warming the entire bottle will reduce the shelf-life of the medium.

2. Cells should be cultured until reaching >85% confluence, at which point they should be either passaged or cryopreserved.

VI. Subculturing

NOTE: The following instructions are for a 100 mm dish. Adjust all volumes accordingly for other size culture vessels.

Preparation for subculturing the first flask:

1. Subculture the cells when they are >85% confluent.
2. For each 100 mm dish of cells to be subcultured:
   a. Thaw 3.0 mL of Trypsin/EDTA and allow to come to room temperature.
   b. Allow 3.0 mL of PBS to come to room temperature.
   c. Allow 5.0 mL of complete medium to come to room temperature.
   d. Remove aliquots of growth medium from 4°C storage and allow to start warming to room temperature.
   e. Prepare new culture vessels.
3. Subculture one flask or dish at a time. All flasks following the first flask will be subcultured following an optimization of this protocol based on calculated cell count, cell viability, and seeding density.

In a Sterile Field:

1. Aspirate the medium from one culture vessel.
2. Rinse the cells with 3.0 mL of room temperature PBS twice. **Do not forget this step.** The medium contains complex proteins and calcium that neutralize the trypsin.
3. Cover the cells with 3.0 mL of Trypsin/EDTA solution.
4. Examine the cell layer microscopically.
5. Allow the trypsinization to continue until approximately 90% of the cells are rounded up. This entire process takes approximately 2 to 6 minutes.
6. At this point, rap the flask against the palm of your hand to release the majority of cells from the culture surface. If only a few cells detach, additional trypsinization may be required. Wait 30 seconds and rap again. If cells still do not detach, wait and rap every 30 seconds thereafter.
7. If the majority of cells do not detach within 7 minutes, the trypsin is either not warm enough or not active enough to release the cells. Harvest the culture vessel as described above, and either re-trypsinize with fresh, warm Trypsin/EDTA solution or rinse with complete medium and then add fresh, warm complete medium to the culture vessel and return to an incubator until fresh trypsinization reagents are available.
8. After cells are released, neutralize the trypsin in the flask with 5.0 mL of room temperature complete medium.
9. Quickly transfer the detached cells to a sterile 50 mL conical tube and place on ice.
10. Rinse the culture vessel with a final 5 mL of complete medium to collect residual cells and add this rinse to the conical tube.
11. Examine the harvested culture vessel under the microscope to ensure the harvest was successful and less than 5% of the cells remain attached.
12. Centrifuge the harvested cells at 200 x g for 5 minutes to pellet the cells.
    a. Aspirate most of the supernatant, allowing 100-200 µl to remain.
    b. Flick the cryovial with your finger to loosen the pellet.
13. Dilute the cells in 5-10 mL of growth medium and note the total volume of the diluted cell suspension.
14. Determine cell count and viability. Make a note of your cell yield for later use.
15. If necessary, dilute the suspension with complete medium to achieve the desired “cells/mL” and re-count the cells.
16. Use the following equation to determine the total number of viable cells.

$$\text{Total \# of Viable Cells} = \frac{\text{Total cell count} \times \text{percent viability}}{100}$$

Determine the total number of culture flasks or wells to inoculate by using the equation below. The number of flasks needed depends upon cell yield and seeding density.

$$\text{Num. of vessels} = \frac{\text{Total number of viable cells}}{\text{Growth area x seeding density}}$$

17. Use the following equation to calculate the volume of cell suspension to seed into your flasks or wells.

$$\text{Seeding volume} = \frac{\text{Total volume of diluted cell suspension}}{\text{number of vessesl as determined in step 17}}$$

18. Prepare each culture vessel by labeling each vessel with the appropriate information.
19. Carefully transfer growth medium to new culture vessels by adding 1 mL growth medium for every 5 cm^2 surface area of the flask (1 mL/5 cm^2).
20. After mixing the diluted cells with a 5 mL pipet to ensure a uniform suspension, dispense the calculated volume into the prepared subculture flasks.
21. If not using vented caps, loosen caps of flasks. Place the new culture vessels into a 37°C humidified incubator with 5% CO_2.

NOTE: Additional population doublings and subcultures are possible, but growth rate, biological responsiveness and function deteriorate with subsequent passages.

VII. Ordering Information

Cryopreserved Cells (Single donor)

Cat. No.	Product	Size
HLECP2	HLEC	>8 x 10^5 cells/vial

Endothelial Medium (must be purchased separately)

Cat. No.	Product	Size
CC-3162	EGM™-2 BulletKit™ Medium	Kit which contains a 500 mL bottle of EBM™-2 Medium (CC-3156) and EGM™-2 SingleQuots™ Kit (CC-4176).
CC-3156	EBM™-2 Medium	Endothelial Basal Medium-2™ Medium (no growth factors) (500 mL)
CC-4176	EGM™-2 SingleQuots™ Kit	Formulates 500 mL of EBM™-2 Basal Medium to EGM™-2 Growth Medium; contains hEGF, 0.5 mL; VEGF, 0.5 mL; R3-IGF-1, 0.5 mL; Ascorbic Acid, 0.5 mL; Hydrocortisone, 0.2 mL; hFGF-β, 2.0 mL; Heparin 0.5 mL; FBS, 10.0 mL; GA, 0.5 mL

VII. Product Warranty

Cultures have a finite lifespan *in vitro*.

Lonza guarantees the performance of cells only if appropriate media and reagents are used exclusively and the recommended storage and use protocols are followed. Any modifications made to the recommended cell systems including the use of alternative media, reagents or protocols, will void cell and media performance guarantees. If you need assistance in selecting the appropriate media, reagents, or protocol, please contact Lonza Scientific Support.

IX. Quality Control

For detailed information concerning QC testing, please refer to the Certificate of Analysis.

When placing an order or when contacting Scientific Support, please refer to the product numbers and descriptions listed above. For a complete listing of all cell culture products, refer to the Lonza website or our current catalog. To obtain a catalog, additional information or Scientific Support, you may contact Lonza by web, e-mail or telephone. Contact details are listed at the top of this document.

X. Safety Statements

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* diagnostic procedures.

WARNING: CELL PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. All human-sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th ed. If you require further information, please contact your site safety officer or Scientific Support.

HLEC cells are manufactured by Samsara Sciences, Inc., San Diego, CA