HUV-EC-C [HUVEC]

CRL-1730™

Description

HUV-EC-C [HUVEC] is an endothelial cell line that was isolated from the vein of the umbilical cord. This cell line can be used in cardiovascular disease research.
Organism: *Homo sapiens*, human
Cell Type: endothelial cell
Tissue: Umbilical cord; Umbilical vein; Vascular endothelium
Morphology: endothelial
Growth properties: Adherent
Disease: Normal

Storage Conditions

Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local

or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C
Atmosphere: 95% Air, 5% CO_2

Handling Procedures

Unpacking and storage instructions:

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Complete medium:

The base medium for this cell line is F-12K Medium (ATCC 30-2004). To make the complete growth medium, add the following components to 450 mL of the base medium:

- 5 mL of a 10 mg/mL stock heparin solution (prepared from Sigma catalog #H3393) for a final concentration of 0.1 mg/mL heparin in complete growth medium
  - Dissolve 1 g Heparin in 100 mL basal F-12K and filter to make a 10 mg/mL stock solution
- 50 mL fetal bovine serum (FBS; ATCC 30-2020)
- 500 µL of 30 mg/mL Endothelial Cell Growth Supplement (ECGS)

Note: Because of limited stability, the ECGS should be added to an aliquot of the above culture medium fresh prior to seeding or performing fluid changes. Complete media supplemented with the below item expires **7 days** after preparation.

To prepare 30 mg/mL ECGS stock solution, aseptically combine:

- 15 mg Corning™ Endothelial Cell Growth Supplement – ECGS (ECGS; Fisher Scientific cat# CB-40006)
- 500 µL F-12K (ATCC 30-2004)

Store in working aliquots at -20°C. ECGS is stable for 1 month when prepared and stored as directed. Do not repeat freeze/thaw.

Handling Procedure:

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete

culture medium. and spin at approximately 125 x g for 5 to 7 minutes.

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). and dispense into a new culture flask at a seeding density of 2.0 x 10^4 to 4.0 x 10^4 viable cells/cm^2. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6). pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet

Subculturing procedure:

Volumes are given for a 75 cm^2 flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes. Corning T-75 flasks (catalog #430641U) are recommended for subculturing this product.

Note: A high quality ECGS prepared from bovine neural tissue (BD Biosciences catalog # 354006 or equivalent) should be used to propagate CRL-1730. It is best to initiate the cells with the highest recommended concentration of ECGS. Moderate to heavy debris and numerous floating cells may be routinely observed in cultures of HUV-EC-C cells. Retain the floating cells by gentle centrifugation and add back to the adherent population.

Cultures should be fully fluid changed every 48 hours. The cells should only be allowed to go 72 hours without fluid changing when the density is less than 50% confluent. Perform full fluid changes rather than media additions.

This cell line produces a lot of floaters and debris especially at higher densities. Cells detach before completely filling in to 100% confluence. It is recommended to subculture the cells when 80 to 90% confluent to avoid excessive floaters. Floating cells are viable and if pronounced, they should be spun down and reseeded back into the growing culture.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an

inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:3 is recommended

Medium Renewal: Two to three times per week

Seeding Density: 8.0 x 10^3 to 3.0 x 10^4 viable cells/cm^2

Reagents for cryopreservation: Complete growth medium supplemented with 5% (v/v) DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: HUV-EC-C [HUVEC] (ATCC CRL-1730)

References

References and other information relating to this material are available at www.atcc.org.

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