ATCC

Product Sheet

HUV-EC-C [HUVEC] (ATCC® CRL-1730™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
1

Intended Use

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

Complete Growth 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 440 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
- 5 mL endothelial cell growth supplement (ECGS; BD Biosciences catalog # 354006)
  - Rehydrate 1 vial using 5 mL basal F-12K
- 50 mL fetal bovine serum (FBS; ATCC 30-2020)

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: HUV-EC-C [HUVEC] (ATCC® CRL-1730™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Homo sapiens*, human
Tissue: umbilical vein/vascular endothelium
Disease: normal
Cell Type: endothelial
Morphology: endothelial
Growth Properties: adherent
DNA Profile:
Amelogenin: X
CSF1PO: 11,12
D13S317: 9,11
D16S539: 11,12
D5S818: 11,12
D7S820: 8,12
THO1: 6,9.3
TPOX: 8,11
vWA: 16
Cytogenetic Analysis: Karyology performed for one batch of CRL-1730 in 1996 reflected a hypodiploid human cell line with a modal chromosome number of 45 occurring in 72% of the cells counted, all of which had monosomic N13. The rate of polyploid cells among this population was 15.8%. This karyology differed from earlier work-ups performed on the cells that showed approximately 60% of the cells retained 2 chromosomes 13. The apparent clonal variation in cultures of CRL-1730 (most likely dependent upon passage and growth conditions) has also been noted in STR profiles with unstable alleles at D13S317 allele #9, D13S317 allele #11, and D7S820 allele #12. Other coexisting subclones include those with 46,XX,-11,-13,i(11p),i(11q) and 46,XX,+11,-13 karyotypes. For all karyotypes performed, both X chromosomes appear normal.

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials 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 vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & 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.

Handling Procedure for Frozen Cells

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

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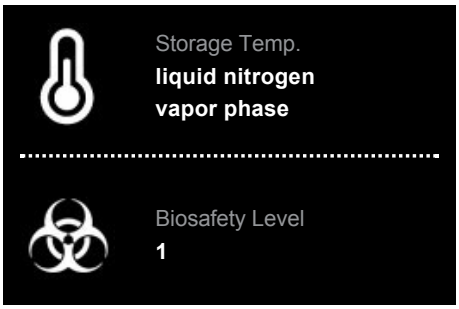

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using the medium described on this product sheet

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached,** aseptically remove all of the growth medium except for approximately 5 to 10 mL to cover the floor of the flask. The old medium can be saved for reuse. Incubate the cells at 37°C in a 5% carbon dioxide gas phase until they are ready to be subcultured.
3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes to spin down the suspended cells into a soft pellet. Remove all but 5 mL of supernatant medium, then resuspend the cells in the remaining medium and add back to a 25 cm^2 flask. The old medium can be saved for reuse. Incubate at 37°C in a 5% carbon dioxide gas phase until they are ready to be subcultured.

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 #430641) 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.

CRL-1730 is a slow growing cell line that has a roughly estimated doubling time of 5 to 6 days. 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

Cryopreservation Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Comments

Endothelial Cell Growth Supplement (ECGS) and unidentified factors from bovine pituitary, hypothalamus or whole brain extracts are mitogenic for this line.

The cells have a life expectancy of 50 to 60 population doublings.

References

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

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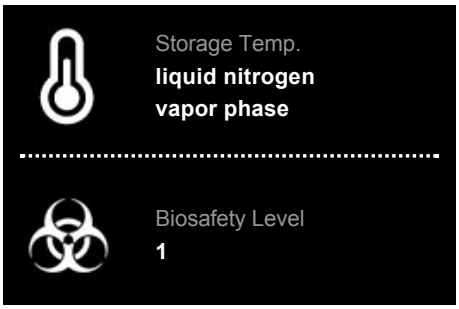

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Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

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Disclaimers

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Additional information on this culture is available on the ATCC web site at www.atcc.org.