THLE-2

CRL-2706™

Description

THLE-2 is an epithelial cell that was isolated from the left lobe of a donor. This cell line was deposited by the National Cancer Institute.

Organism: *Homo sapiens*, human
Cell Type: epithelial cell
Tissue: Liver; Left lobe
Age: adult
Morphology: epithelial
Growth properties: Adherent
Patent depository: This material was deposited with the ATCC Patent Depository to fulfill U.S. or international patent requirements. This material may not have been produced or characterized by ATCC. As an International Depository Authority (IDA) for patent deposits, ATCC is required to complete viability testing only at time of initial deposit of patent material. Patent deposits are made available on behalf of the Depositor when the pertinent U.S. or international patent is issued, but material may not be used to infringe the patent claims.
Patent number:
5,759,765
Technical information: ATCC Product Experience does not have technical information on patent deposits that are not produced or characterized by ATCC. Additional information can be found in the corresponding patent available from the patent holder or with the U.S. and/or international patent office.

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 2

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.

Cells contain SV40 sequences

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:

BEGM from Lonza/Clonetics Corporation, Walkersville, MD 21793 (BEGM Bullet Kit; CC3170). The kit includes 500 mL basal medium and separate frozen additives from which we discard the gentamycin/ Amphotericin (GA) and Epinephrine and to which we add extra 5 ng/mL EGF, 70 ng/mL Phosphoethanolamine and 10% fetal bovine serum.

Note: The prepared kit with the additional supplements has a shelf life of 1 month following preparation.

Because of limited stability, the EGF should be added to an aliquot of the above culture medium fresh prior to seeding or performing fluid changes. Complete medium with the below components can be stored for a maximum of 10 days. This applies to previously supplemented media stock and/or media inside vessels in culture

Prepare a 5 ug/mL stock human EGF: Use 1ug per 1 mL culture media

Store 5 ug/mL stock human EGF in working aliquots at -20'C. EGF is stable for 3 months when prepared and stored as directed. Do not repeat freeze/thaw.

Handling Procedure:

Note: The flasks used should be precoated with a mixture of 0.01 mg/mL fibronectin, 0.03 mg/mL bovine collagen type I and 0.01 mg/mL bovine serum albumin dissolved in BEBM medium.

When ready to use coated flask, gently aspirate the coating solution, loosen the cap and leave in the BSC to air dry for 10-15 minutes. Use the appropriate DPBS volume to rinse 3 times and air dry vertically for 10-15 minutes.

Note: Do not dispense DPBS directly onto the coated surface and gently tilt the vessel(s) to rinse. Aspirate remaining liquid, if present

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 150 to 400 x g for 8 to 12 minutes (280 x g for 10 minutes). Discard supernatant.
4. Resuspend the cell pellet with the recommended complete medium and at the seeding density recommended on the Certificate of Analysis. (2.0×10^4 to 6.0×10^4 viable cells/cm^2) and dispense into a 25 cm^2 or a 75 cm^2 culture flask. 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).
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 used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

Note: The flasks used should be **precoated** with a mixture of 0.01 mg/mL fibronectin, 0.03 mg/mL bovine collagen type I and 0.01 mg/mL bovine serum albumin dissolved in BEBM medium.

1. Remove and discard culture medium.
2. Add a fresh 0.05% trypsin-EDTA solution (2.0 – 3.0 mls for a T75 flask) to cover the cells, rinse and remove most of the trypsin solution leaving a thin coating behind..
3. Allow the culture to sit at room temperature (or 37°C) until the cells detach 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. Neutralize the trypsin with 0.1% soybean trypsin inhibitor and aspirate cells by gently pipetting. Remove the dissociation agent by gentle centrifugation (150 x g to 400 x g for 8-12 minutes) and resuspend cells in fresh complete growth medium.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.
7. A 0.05% Trypsin-EDTA solution (GIBCO cat# 25300-054) is recommended for dissociation. Neutralize with a 0.1% Soybean Trypsin inhibitor solution (ATCC 30-2104).

Subculture Ratio: 1:3 to 1:6
Medium Renewal: Two to three times a week.
Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in **Culture of Animal Cells, a manual of Basic Technique** by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.
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: THLE-2 (ATCC CRL-2706)

References

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

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