THLE-3 (ATCC® CRL-11233™)

Storage Temp.
liquid nitrogen
vapor phase

Biosafety Level
2

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

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.

Cytogenetic Analysis: near diploid

DNA Profile:
Amelogenin: X
CSF1PO: 11, 12
D13S317: 13
D16S539: 11, 12
DSS818: 13
D7S820: 8, 10
THO1: 8, 9.3
TPOX: 6, 9
vWA: 17, 18

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.

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. 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 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
If the cells are still attached, aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere 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. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to pre-coated 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

Subculturung Procedure

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

The flasks should be pre-coated 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. Briefly rinse the cell layer with 0.05% (w/v) Trypsin-53mM EDTA solution (GIBCO cat# 25300-054) 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 with 5 to 15 minutes). Note: To avoid agitating 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 0.1% Soybean Trypsin inhibitor and aspirate cells by gently pipetting.
5. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new coated culture vessels.
7. Place culture vessels in incubators at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended

Medium Renewal: Every 2 to 3 days

Flask Coating

1. Prepare a mixture of 0.01 mg/mL fibronectin, 0.03 mg/mL bovine collagen type I and 0.01 mg/mL bovine serum albumin (BSA) dissolved in culture medium. Store pre-prepared Coating Solution at 4°C in cold room for up to 3 months.
2. For a growth area of 75 cm², add 4.5 mL of the fibronectin/collagen/BSA solution and rock gently to coat the entire surface.
3. Incubate the freshly coated vessel(s) in a 37°C incubator overnight (it is preferable to use tissue culture vessels with tightened, plug-seal caps to prevent evaporation during the coating process).
4. Store coated flasks with solution at room temperature, light protected, up to 1 month. Suction off solution before plating cells.

Cryopreservation Medium

Complete culture medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

The virus was generated by introducing a retroviral vector containing the oval of Bgl I-Hpa I fragment of SV40 T antigen into the amphotropic packaging cell line PA317.

THLE-2 and THLE-3 cells express phenotypic characteristics of normal adult liver epithelial cells. They are non-tumorigenic when injected into athymic nude mice, have near-diploid karyotypes, and do not express alpha-fetoprotein.

THLE-2 and THLE-3 cells metabolize benzo[a]pyrene, N-nitrosodimethylamine, and aflatoxin B1 to their ultimate carcinogenic metabolites that adduct DNA, which functions cytotoxic p540 pathways. Other enzymes involved in metabolism of chemical carcinogens, such as epoxide hydrolase, NADPH cytochrome P450 reductase, superoxide dismutase, catalase, glutathione S-transferases, and glutathione peroxidase are also retained by THLE cells.

A culture submitted to the ATCC in January of 1993 was found to be contaminated with mycoplasma. Progeny were cured by a 21-day treatment with mycoplasma removal agent (MRA). The cells were assayed for mycoplasma, by the Hoechst stain, PCR and the standard culture test, after a six-week period following treatment. All tests were negative.
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.

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