Cell Line Designation: THLE-2

AddexBio Catalog No. T0015001 (formerly C0015003)

Cell Line Description:

Disease: Normal, Immortalized human liver cells

Origin: The THLE-2 cell line was derived from primary normal liver cells by infection with SV40 large T antigen.

Species: Homo sapiens

Tissue: Liver/left lobe

Properties: Epithelial; adherent

Patient: Adult

Complete Medium: BEGM Bullet Kit; CC3170 from Lonza + 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% FBS.

Subculture 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. Briefly rinse the cell layer with 0.05% (w/v) Trypsin-0.53% (w/v) EDTA solution (GIBCO cat# 25300-054) to remove all traces of serum that 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.

AddexBio Technologies
4907 Morena Blvd, Ste 1408
San Diego, CA 92117 USA
858-348-7819
Fax: 858-538-8847
Email:customersupport@addexbio.com
www.addexbio.com

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 with 5% CO_2.

Subculture Ratio: 1:3 to 1:6

Medium Renewal: Add fresh medium every 3 to 4 days.

Freezing Medium: Complete culture medium supplemented with 5% (v/v) DMSO

Additional Information: Additional product and technical information can be obtained from the catalog references and the Addexbio Technical Information site at www.addexbio.com, or by email at customersupport@addexbio.com.

Biosafety Level: 2 [Cells contain SV40 viral DNA sequences]

Appropriate safety procedures should always be used with this material. laboratory safety is discussed in the following publication: Biosafety in Microbiological and Biomedical Laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is also available online at www.cdc.gov/od/ohs/biosafty/bmbl4/bmbl4toc.htm

Use Restrictions: **These cells are distributed for research purposes only.** Addexbio does not recommend third party distribution of this cell line, as this practice has resulted in the unintentional spreading of contaminated cell lines.

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.

Safety Precaution:

Addexbio 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

AddexBio Technologies
4907 Morena Blvd, Ste 1408
San Diego, CA 92117 USA
858-348-7819
Fax: 858-538-8847
Email:customersupport@addexbio.com
www.addexbio.com

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.

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 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 the centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125xg for 5 to 7 minutes.

4. Resuspend cell pellet with the recommended complete medium and dispense into a new 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 (7.0-7.6).

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended.

References for THLE-2 cells:

Pfeifer AM, Cole KE, Smoot DT, Weston A, Groopman JD, Shields PG, Vignaud JM, Juillerat M, Lipsky MM, Trump BF, et al. Simian virus 40 large tumor antigen-immortalized normal human liver epithelial cells express hepatocyte characteristics and metabolize chemical carcinogens. Proc Natl Acad Sci USA. 1993;90(11):5123-5127.

rev. 06/2017
AddexBio Technologies
4907 Morena Blvd, Ste 1408
San Diego, CA 92117 USA
858-348-7819
Fax: 858-538-8847
Email:customersupport@addexbio.com
www.addexbio.com

Lot Specific Information Sheet for AddexBio Cat #: T0015003

Lot Number: 1476382

Designation: THLE-2 CELLS

Total Cells/mL: >1.3x10^6

Expected Viability: 65.0-69.2%

Ampule Passage #: 13

Dilute Ampule Content: 1:10 (T-25) or 1:15 (T-75)

Volume/Ampule: 1 mL

AddexBio Technologies
4907 Morena Blvd, Ste 1408
San Diego, CA 92117 USA
858-348-7819
Fax: 858-538-8847
Email:customersupport@addexbio.com
www.addexbio.com