



T HESCs

CRL-4003™

Description

T HESCs are hTERT-immortalized cells exhibiting fibroblast morphology that were isolated from the uterus of a female patient with non-malignant myomas. This cell line has applications in drug development, high-throughput screening, and quality control.

Organism: *Homo sapiens*, human

Cell Type: fibroblast

Tissue: Uterus; Endometrium

Age: adult

Gender: Female

Morphology: Fibroblast-like

Growth properties: Adherent

Disease: Non Malignant Myomas

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

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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 cytomegalovirus (CMV) DNA 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% CO₂

Handling Procedures

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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: A 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium with 3.1 g/L glucose and 1mM sodium pyruvate and without phenol red (Sigma Cat# D 2906) supplemented with 1.5 g/L sodium bicarbonate, 1% ITS+ Premix (BD Cat# 354352), 500ng/mL puromycin, 90%; charcoal/dextran treated fetal bovine serum (HyClone Cat# SH30068.03), 10%

Handling Procedure: To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If storage of the frozen culture is necessary upon arrival, store the vial in liquid nitrogen vapor phase and NOT at -70°C . Storage at -70°C will result in loss of viability.

1. Prepare a 25 cm² or a 75 cm² culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents, the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.
2. 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).
3. 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 operations from this point on should be carried out under strict aseptic conditions.
4. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge the cell suspension at approximately 125 x g for 5 to 7 minutes.
5. Discard the supernatant and resuspend the cells in fresh growth medium (see the batch-specific information for the recommended dilution ratio). Add this suspension to the prepared culture vessel.
6. Incubate the culture at 37°C in a suitable incubator.
7. A 5% CO₂ / 95% air atmosphere is recommended if using the medium described on this product sheet.

Subculturing procedure:

Volumes are given for T-75 flasks; proportionally reduce or increase the amount of dissociation medium for culture vessels of other sizes.

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1. Remove and discard culture medium.
2. Add 2.0-3.0 mL of 0.25% Trypsin-0.53mM EDTA solution to the flask and observe cells under an inverted microscope until the 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 to detach may be placed at 37°C to facilitate dispersal.
3. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
4. Transfer cell suspension to a centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
5. Discard supernatant and resuspend cells in fresh growth medium.
6. Add appropriate aliquots of cell suspension to new culture vessels. An inoculum of 3×10^3 to 4×10^3 viable cells/cm² is recommended. Subculture when cell concentration reaches between 2×10^4 and 3×10^4 cells/cm².
7. Incubate cultures at 37°C.

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

Medium Renewal: Every 2 to 3 days

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual of Basic Techniques** by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

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: T HESCs (ATCC CRL-4003)

References

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

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