hTERT Dermal Microvascular Endothelial Cell, Neonatal

CRL-4060™

Description

Organism: *Homo sapiens*, human
Tissue: Skin; Dermis
Gender: Male
Morphology: epithelial-like
Growth properties: Adherent
Disease: Normal
Cells per vial: ≥ 1.5 x 10^6
Volume: 1.0 mL

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.

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: The base medium for this primary cell is Vascular Cell Basal medium (ATCC PCS-100-030). To make the complete medium add the following components to the base medium:

- Microvascular Endothelial Cell Growth Kit – BBE (ATCC PCS-110-040)
- 0.5 µg/mL puromycin (10 mg/ml stock, Gibco, A1138-03)

Handling Procedure:

To ensure 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 at 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 10.0 mL complete medium and mix well by pipetting.
4. Centrifuge cells at 220 x g for 5 minutes.
5. Re-suspend cells in 5 mL complete media.
6. Take an aliquot of cells for cell viability and concentration
7. Seed an appropriate sized flask with remaining cell suspension at a density of 3.0 X 10_3 cells/cm_2, gently rock to evenly distribute cells, then 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. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

1. Subculture cells when around 80% confluent. Remove and discard culture

medium.
2. Briefly rinse the cells with D-PBS (ATCC 30-2200).
3. Add 2.0 to 3.0 mL of Trypsin-EDTA for Primary Cells Solution (ATCC PCS-999-003) to flask, ensure complete coverage of cells and remove trypsin, then incubate and observe cells until cells have detached (usually within 2 to 3 minutes).
4. Once detached, add 2.0 to 3.0 mL of soybean trypsin inhibitor (ATCC 30-2104) and aspirate to suspend cells. Transfer cell suspension into a 15ml conical tube.
5. Add 4.0 to 7.0 mL of complete growth medium to flask to wash and recover residual cells, aspirate cells by gently pipetting. Transfer suspension into previous 15ml conical tube.
6. Collect cells by centrifugation at 220xg for 5min.
7. Remove supernatant and resuspend cell pellet in complete medium.

Interval: Maintain cultures at a cell concentration between 5.0 X 10^3 and 5.0 X 10^4 cell/cm^2.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:10 is recommended
Medium Renewal: 2 to 4 times per week
Reagents for cryopreservation: Complete growth medium supplemented with 10% (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: hTERT Dermal Microvascular Endothelial Cell, Neonatal (ATCC CRL-4060)

References

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

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Revision

This information on this document was last updated on 2024-10-25

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