hTERT-immortalized Dermal Melanocyte

CRL-4059™

Description

hTERT-immortalized Dermal Melanocyte cells were isolated from a normal female. They were immortalized using the catalytic subunit of the human telomerase (hTERT) gene. This product has applications in drug development for melonoma, psoriases, cosmetic research, and other skin diseases.

For-profit customers intending to use this product for non-commercial screening must include the one-time "ATCC Screening Fee" (ATCC® ACS-2103F) with their first purchase of this product.

Organism: *Homo sapiens*, human
Tissue: Skin
Gender: Female
Growth properties: Adherent
Disease: Normal
Cells per vial: Approximately 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 cell is Dermal Cell Basal Medium (485 mL; ATCC PCS-200-030). To make the complete medium add the components of the Melanocyte Growth Kit (ATCC PCS-200-042) and Puromycin at a concentration of 0.5 µg/mL.

The recovery media (without Puromycin) consists of the base medium for this cell line is Dermal Basal Medium (ATCC PCS-200-030), the components of the Melanocyte Growth Kit (ATCC PCS-200-042), and 40% FBS (without puromycin).

Contents of Melanocyte Growth Kit

- rh Insulin, 0.5 mL (5 µg/mL final concentration)
- Ascorbic Acid, 0.5 mL (50 µg/mL final concentration)
- L-Glutamine, 15.0 mL (6 mM final concentration)
- Epinephrine, 0.5 mL (1.0 µM final concentration)
- Calcium Chloride, 840 µL (1.5 mM final concentration)
- Peptide Growth Factor, 1.0 mL
- M8 Supplement, 5 mL

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. Prepare 8 to 10 ml of MEL Medium **supplemented with 40% FBS and without puromycin**. Place recovery medium into a T25 flask and equilibrate to temperature and pH for 30 minutes in a 37°C, 5% CO_2 incubator prior to thawing cells.
2. Thaw vial 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 of the

operations from this point on should be carried out under strict aseptic conditions.

4. Transfer the vial contents to a centrifuge tube containing 1.0 mL of equilibrated recovery medium, which includes complete medium supplemented with 40% serum. **Do NOT pipette up and down to mix.**
5. Gently rock to evenly distribute cells, then incubate the culture at 37°C in a suitable incubator. A 5% CO2 in air atmosphere is recommended if using the medium described on this product sheet.
6. After 24 hours, replace medium with complete medium supplemented with 0.5ug/ml puromycin.

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. Remove and discard culture medium.
2. Briefly rinse the cell layer with PBS (ATCC 30-2200) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA for Primary Cells Solution 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 a 0.05% soybean trypsin inhibitor and aspirate 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 150xg for 5min.
7. Resuspend cell pellet in 1.5 to 3.0 mL of complete medium. Add appropriate aliquots of the cell suspension to new culture vessels.
   Cultures should be established from cryopreservation between 2 x 10^4 and 3 x 10^4 viable cells/cm^2.
8. Incubate cultures at 37°C.

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: 3 times per week

Reagents for cryopreservation: 100% Stem Cell Freeze Medium (ATCC ACS-3020)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: hTERT-immortalized Dermal Melanocyte (ATCC CRL-4059)

References

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

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