hTERT Neonatal Dermal Melanocytes

CRL-4064™

Description

hTERT-immortalized Neonatal Melanocytes (ATCC® CRL-4064™) were created by selecting a single cell clone from primary melanocytes that had been retrovirally transduced with the gene encoding the human telomerase catalytic subunit (hTERT). Essential melanocyte character post-transduction was confirmed by immunofluorescence staining for the melanocyte marker TRP1. The CRL-4064 cell line also retains typical melanocyte morphology as observed through phase contrast microscopy.

Organism: *Homo sapiens*, human
Tissue: Skin
Age: neonate
Gender: Male
Morphology: Fibroblast-like
Growth properties: Adherent
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

Complete medium:

The base medium for these cells is Dermal Cell Basal Medium (ATCC PCS-200-030). To make the complete medium, add the components of Adult Melanocyte Growth Kit (ATCC PCS-200-042) according to manufacturer instructions.

It's important to note that CRL-4064 requires two distinct media formulations:

- **Recovery/Thawing medium:** Dermal Cell Basal Medium (PCS-200-030) + Adult Melanocyte Growth Kit (PCS-200-042) + 30% FBS

  Prepare **Recovery/Thawing** medium by adding 12 mL FBS (ATCC 30-2020) to 28 mL of the Complete medium

  Note: The above media is stable for 4 weeks at 4 to 8°C.

  FBS should not be heat inactivated as this can reduce or destroy serum growth factors, attachment factors and hormones.

- **Growth medium:** Dermal Cell Basal Medium (PCS-200-030) + Adult Melanocyte Growth Kit (PCS-200-042) + 0.5 µg/mL puromycin

  Prepare **Growth medium** by adding 24 uL Gibco Puromycin (10 mg/mL; Thermofisher Scientific catalog # A11138-03 or equivalent) per 480.3 mL Complete medium.

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. Use 8–10 mL of prepared **Recovery/Thawing** medium and place it in a T25 flask. Equilibrate the medium in a 37°C, 5% CO_2 incubator for 30 minutes before cell thawing.
2. Thaw the cryovial rapidly in a 37°C water bath, keeping the O-ring and cap dry. Thawing should take no more than 2 minutes.
3. Before the last ice crystal has melted, promptly remove the vial from the water bath. This helps prevent osmotic shock and temperature stress that may compromise cell viability.

4. Immediately disinfect the outside of the vial with 70% ethanol. From this point forward, proceed under strict aseptic conditions
5. Transfer the vial contents into a centrifuge tube containing 1.0 mL of equilibrated thawing medium. **Do NOT pipette up and down to mix.** Rinse the vial with an additional 1.0 mL of thawing medium and add to the same tube. **Do NOT pipette up and down to mix.**
6. Centrifuge at approximately 300 x g for 8 minutes, then remove the cryoprotectant. Resuspend cell pellet into Recovery/Thawing medium. Incubate the culture at 37°C and 5% CO2
7. After 24 hours, replace medium with **Growth medium** (complete medium supplemented with 0.5ug/ml puromycin) and incubate the culture at 37°C and 5% CO2

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 2.0 x 10^4 and 3.0 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 Neonatal Dermal Melanocytes (ATCC CRL-4064)

References

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

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