TIME

CRL-4025™

Description

TIME are hTERT-immortalized cells exhibiting endothelial-like morphology that were isolated from a primary culture of neonatal foreskin microvascular endothelial cells (HMVEC) of the dermis. This product has applications for drug development and cardiovascular disease research.

Cell Type: endothelial cell
Tissue: Skin; Dermal microvascular endothelium; Foreskin
Age: neonate
Gender: Male
Morphology: Endothelial-like
Growth properties: Adherent
Disease: Normal

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.

Cells contain Encephalomyocarditis virus (EMCV) 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% 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 line is Vascular Cell Basal Medium (ATCC® PCS-100-030), supplemented with Microvascular Endothelial Cell Growth Kit-VEGF (ATCC® PCS-110-041) and 12.5 µg/mL blasticidine.

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 cm^2 or a 75 cm^2 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% CO_2/95% 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 flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

Note: Subculture when cultures are about 80% confluent.

1. Prior to subculturing, determine the number of flasks needed. Add the

appropriate volume of medium to each flask and allow the flasks to equilibrate in a 37°C, 5% CO_2, humidified incubator for at least 30 minutes. If not using vented caps, loosen caps of flasks.

2. Remove and discard spent medium.
3. Briefly rinse the cells with Dulbecco's Phosphate Buffered Saline (D-PBS, ATCC 30-2200) and discard rinse solution.
4. Add 2.0 to 3.0 mL room temperature Trypsin-EDTA for Primary Cells (ATCC PCS-999-003) to the flask. Incubate at 37°C for 5 min (until cells have detached).
5. Neutralize trypsin by adding an equal volume of room temperature 2% FBS in D-PBS.
6. Transfer cells to a centrifuge tube. Rinse the flask with an additional room temperature 2% FBS in D-PBS and pool into centrifuge tube with cells.
7. Centrifuge cells at 250 x g for 10 min at room temperature.
8. Remove supernatant. Resuspend pellet in 6.0 to 8.0 mL Complete Growth Medium.
9. Count cells, and seed 5 x 10^3 to 8 x 10^3 viable cells/cm^2 to new culture vessels. Subculture when cells become 80 to 90% confluent, which normally yield approximately 3.0 x 10^4 viable cells/cm^2.
10. Incubate cultures at 37°C in a 5% CO_2 humidified incubator.

Subcultivation ratio: A subcultivation ratio of 1:4 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 Technique** by R. Ian Freshney.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: TIME (ATCC CRL-4025)

References

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

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