



TIME-GFP

CRL-4045™

Description

Organism: *Homo sapiens*, human

Cell Type: microvascular endothelial cell

Tissue: Skin; Foreskin

Age: neonate

Gender: Male

Morphology: endothelial

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

TIME-GFP

CRL-4045

or national agencies.

Cells contain SV40 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

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

TIME-GFP

CRL-4045

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-BBE (ATCC PCS-110-040) OR Microvascular Endothelial Cell Growth Kit-VEGF (ATCC PCS-110-041). Add Blasticidin (Life Technologies Cat. No. A11139-03) at a final concentration of 12.5 ug/mL and G418 (Life Technologies Cat. No. 11811-023) at a final concentration of 200 ug/mL. Note: Do not filter 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 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.

Subculturing procedure: Volumes used in this protocol are for 75 cm² flasks; proportionally reduce or increase amount of dissociation solutions for culture vessels of other sizes.

Subculture when the culture is about 90% confluent.

1. Remove and discard spent medium.
2. Briefly rinse the cells with Dulbecco's Phosphate Buffered Saline (DPBS, ATCC

TIME-GFP

CRL-4045

- 30-2200) and discard rinse solution.
3. 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).
 4. Neutralize trypsin by adding an equal volume of room temperature 2% FBS in DPBS.
 5. Centrifuge cells at 250 x g for 5 min at room temperature.
 6. Remove supernatant. Resuspend pellet in 6.0 to 8.0 mL Complete Growth Medium.
 7. Count cells, and seed 5.0×10^3 to 8.0×10^3 viable cells/cm² to new culture vessels.

Medium Renewal: Every 2-3 days.

Reagents for cryopreservation: Fetal bovine serum 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: TIME-GFP (ATCC CRL-4045)

References

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

Warranty

The product is provided 'AS IS' and the viability of ATCC[®] products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet, website, and Certificate of Analysis. For living cultures, ATCC lists the media formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory results, a

TIME-GFP

CRL-4045

change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP standards, typicality, safety, accuracy, and/or noninfringement.

Disclaimers

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. Any proposed commercial use is prohibited without a license from ATCC.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate or complete and the customer bears the sole responsibility of confirming the accuracy and completeness of any such information.

This product is sent on the condition that the customer is responsible for and assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, and use of the ATCC product including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. As a condition of receiving the material, the customer agrees that any activity undertaken with the ATCC product and any progeny or modifications will be conducted in compliance with all applicable laws, regulations, and guidelines. This product is provided 'AS IS' with no representations or warranties whatsoever except as expressly set forth herein and in no event shall ATCC, its parents, subsidiaries, directors, officers, agents, employees, assigns, successors, and affiliates be liable for indirect, special, incidental, or consequential damages of any kind in connection with or arising out of the customer's use of the product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, ATCC is not liable for

damages arising from the misidentification or misrepresentation of such materials.

Please see the material transfer agreement (MTA) for further details regarding the use of this product. The MTA is available at www.atcc.org.

Copyright and Trademark Information

© ATCC 2023. All rights reserved.

ATCC is a registered trademark of the American Type Culture Collection.

Revision

This information on this document was last updated on 2022-12-21

Contact Information

ATCC

10801 University Boulevard

Manassas, VA 20110-2209

USA

US telephone: 800-638-6597

Worldwide telephone: +1-703-365-2700

Email: tech@atcc.org or contact your local distributor
