



# NF $\kappa$ B-TIME

CRL-4049™

## Description

**Organism:** *Homo sapiens*, human

**Cell Type:** microvascular endothelial cell

**Tissue:** Skin; Foreskin

**Age:** neonate

**Gender:** Male

**Morphology:** endothelial

**Growth properties:** Adherent

**Disease:** Normal

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## Storage Conditions

**Product format:** Frozen

**Storage conditions:** Vapor phase of liquid nitrogen

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## 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.

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## 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

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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.

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## Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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## Growth Conditions

**Temperature:** 37°C

**Atmosphere:** 95% Air, 5% CO<sub>2</sub>

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## 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

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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  $\mu$ g/mL and Hygromycin (Life Technologies Cat. No. 10687-010) at a final concentration of 20  $\mu$ g/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  $-70^{\circ}\text{C}$ . Storage at  $-70^{\circ}\text{C}$  will result in loss of viability.

1. Prepare a 25 cm<sup>2</sup> or a 75 cm<sup>2</sup> 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 cm<sup>2</sup> flasks; proportionally reduce or increase amount of dissociation solutions for culture vessels of other sizes.

Subculture when the culture is about 90% confluent.

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1. Remove and discard spent medium.
2. Briefly rinse the cells with Dulbecco's Phosphate Buffered Saline (DPBS, ATCC 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  $5.0 \times 10^3$  to  $8.0 \times 10^3$  viable cells/cm<sup>2</sup> 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)

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## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: NFκB-TIME (ATCC CRL-4049)

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## References

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

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