



Product Sheet

A-549 VIM RFP (ATCC® CCL-185EMT™)

Please read this FIRST



Storage Temp.
liquid nitrogen
vapor phase



Biosafety Level
2

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth Medium

The base medium for this cell line is F-12K Medium (ATCC 30-2004). To make the complete medium add the following to the base medium:

- Fetal Bovine Serum (FBS; ATCC 30-2020) for a final concentration of 10%

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: A-549 VIM RFP (ATCC® CCL-185EMT™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Homo sapiens*, human
Tissue: lung
Disease: carcinoma
Cell Type: epithelial
Age: 58 years
Gender: male
Morphology: epithelial-like
Growth Properties: adherent
DNA Profile:
Amelogenin: X,Y
CSF1PO: 10,12
D13S317: 11
D16S539: 11,12
D5S818: 11
D7S820: 8,11
THO1: 8,9,3
TPOX: 8,11
vWA: 14

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials 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 vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & 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.

Handling Procedure for Frozen Cells

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. Initial seeding density is 1×10^4 and 2×10^4 . 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).
2. 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.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium. and spin at approximately $125 \times g$ for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6). pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Subculturing Procedure

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Corning® T-75 flasks (catalog #430641) are recommended for



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subculturing this product.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
Cultures can be established between 2×10^3 and 1×10^4 viable cells/cm². Do not exceed 7×10^4 cells/cm².
6. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 6×10^3 and 6×10^4 cell/cm².

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended

Medium Renewal: 2 to 3 times per week



Cryopreservation Medium

Complete growth medium plus 5% DMSO



Comments

In lung cancer, Vimentin intermediate filament (IF) proteins have been implicated in many aspects of cancer initiation and progression, including tumorigenesis, epithelial-to-mesenchymal transition (EMT/MET), and the metastatic spread of cancer. Vimentin expression is generally upregulated when the cell is in the mesenchymal relative to the epithelial status. Here, we created a Vim-RFP reporter cell line (CCL-185EMT) using the CRISPR/Cas9 gene editing platform and the parental A549 (CCL-185) non-small cell lung cancer (NSCLC) cell line (a gold standard for studying EMT in cancer metastasis within the lung cancer research community). The created CCL-185EMT cell line harbors a C-terminal red fluorescent protein (RFP) tag on the vimentin gene. This will enable the tracking of the EMT status of cells in vitro by monitoring RFP expression. The integrity of the Vim RFP knock-in has been verified at the genomic, mRNA and protein level for sequence and expression. Functional evaluation of CCL-185EMT shows sensitivity to anti-EMT drugs PP1 and A83-1. This provides the foundation for the high throughput (HTS) identification of new anti-EMT drugs for metastatic NSCLC therapy. The A549 Vim RFP reporter cell line provides a visualizable, convenient and sensitive platform for research on the mechanisms of metastasis in vitro and the development of new antitumor drugs for metastatic NSCLC.



References

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



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Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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confirmed to be accurate.



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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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