Description

Organism: Homo sapiens, human
Tissue: skin
Disease: malignant melanoma
Age: 54 years
Gender: female
Morphology: epithelial
Growth Properties: adherent
DNA Profile:
Amelogenin: X
CSF1PO: 11,12
D13S317: 11,14
D16S539: 9
D5S818: 12
D7S820: 9
TH01: 8
TPOX: 8,10
vWA: 16,17

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. 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 150 x 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% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for...
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 sub culturing this product.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-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).
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.
6. Incubate cultures at 37°C.

**Interval**: Maintain cultures at a cell concentration between 8 X 10³ and 4 X 10⁴ cells/cm².

**Subcultivation Ratio**: A subcultivation ratio of 1:2 to 1:6 is recommended.

**Medium Renewal**: 2 to 3 times per week

Cryopreservation Medium

Complete growth medium supplemented with 5% (v/v) DMSO (ATCC 4-X)

Comments

This is a malignant BRAFV600E/MEK1Q56P mutant isogenic line derived from the parental A375 (ATCC CRL-1619) cell line. The c.167A>C knock-in mutation encoding MEK1 p.Q56P protein expression was generated at ATCC by utilizing the CRISPR/Cas9 gene editing technology. This is a heterozygous mutation expressing the MEK1 wild-type and the c.167A>C mutant alleles.

BRAF is a proto-oncogene encoding B-RAF, a serine/threonine kinase of the RAF family that acts downstream of RAS and upstream of MEK in the MAPK/ERK signaling pathway. Mutations in BRAF lead to excessive cellular proliferation, differentiation, and survival. BRAF V600E mutations are present in 50% of melanomas and although there are current BRAF inhibitors used as successful therapeutics, patients often become resistant to drugs several months following treatment. One mechanism of resistance to these inhibitors is caused by upstream secondary RAS acquired mutations. The MEK1 Q56P mutant isogenic line, ATCC CRL-1619IG-3 has been validated at the genomic, transcript, and protein bio-functional levels and exhibits significant resistance to the BRAF inhibitors Dabrafenib and Vemurafenib, as well as the MEK inhibitor trametinib when compared to its parental cell line. Furthermore, A-375 MEK1Q56P (ATCC CRL-1619IG-3™) cells display increased sensitivity to combination MEK/BRAF inhibitor treatments, making this line an ideal model system for the development of novel combination therapies targeting multiple points in the RAS-RAF-MEK-ERK-MAPK signaling pathway, as well as for the screening of new potential BRAF and MEK inhibitors.

References

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

Biosafety Level: 2

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 US Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.
ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans. 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.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure 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 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.

© ATCC 2019. All rights reserved. ATCC is a registered trademark of the American Type Culture Collection. [02/12]