ATCC

Product Sheet

M4A4 LM3-2 GFP (ATCC® CRL-2916™)

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 ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to 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: M4A4 LM3-2 GFP (ATCC® CRL-2916™)

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

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Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Homo sapiens*, human
Disease: cancer
Cell Type: melanocyte, Melanoma
Age: 31
Gender: female
Morphology: epithelial
Growth Properties: adherent
DNA Profile:
Amelogenin: X
CSF1PO: 11
D13S317: 12
D16S539: 13
D5S818: 11,12
D7S820: 8,10
THO1: 6,7
TPOX: 8,11
vWA: 16,18

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

Handling Procedure for Frozen Cells
To insure 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.
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.
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 125 x g for 5 to 10 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm^2 or a 75 cm^2 culture flask. 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).
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.

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Subculturing Procedure

Protocol: To avoid phenotypic drift it is recommended to make frozen aliquots of the cells and use each aliquot for only 10 passages.

Note: If more than 5-10% non-fluorescing cells reappear at any time it is recommended to use 600 micrograms/mL G418 for 2-4 weeks to kill off the revertants. Resistance to higher concentrations of G418 depends on the number of copies of the neo resistance gene that the cell has incorporated which is not uniform for the whole population.

Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 1.0 to 2.0 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 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Transfer cell suspension to a centrifuge tube and spin at approximately 125 X g for 5 to 10 minutes. Discard supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 3 X 10(3) to 5 X 10(3) viable cells/sq. cm is recommended.
7. Incubate cultures at 37C. We recommend that you maintain cultures at a cell concentration between 2 X 10(5) and 3 X 10(5) cells/sq. cm.

Subcultivation Ratio: A subcultivation ratio of 1:20 to 1:40 is recommended
Medium Renewal: 2 to 3 times a week

Cryopreservation Medium

Cryoprotectant Medium
Complete growth medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Comments

The M4A4 LM3-2 GFP (CRL-2916) was derived from a second generating lung metastasis after inoculation of M4A4 GFP cells in a nude mouse mammary gland. M4A4 LM3-4 CL 16 GFP (ATCC CRL-2917) cell line was established from a third generation lung metastasis. The M4A4 GFP (ATCC CRL-2915) was developed by the transduction of the GFP gene into M4A4 (CRL-2914) cell line. The parental cell lines ATCC CRL-2914 (M4A4) and ATCC CRL-2918 (NM2C5) were derived from the human breast cancer cell line, MDA.MB-435. M4A4 is highly metastatic in immuno-deprived mice, while NM2C5 is weakly or virtually non-metastatic. These well characterized, tumorigenic human isogenic cell lines have dramatically opposite metastatic phenotypes and are ideal for metastatic studies.
Note: Recent studies have generated questions about the origin of the parent cell line, MDA-MB-435. Gene expression analysis of the cells produced microarrays in which MDA-MB-435 clustered with cell lines of melanoma origin instead of breast. Additional studies have since corroborated a melanocyte origin of MDA-MB-435, to which ATCC has responded by pursuing its own investigation into the identity of this cell line. The cell line to which MDA-MB-435 is reported to have been cross-contaminated with is the M14 melanoma line.

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 U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

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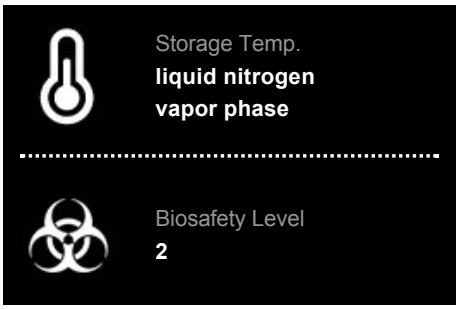

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ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell 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.
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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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