ATCC

Product Sheet

M059K (ATCC® CRL-2365™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
1

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

These cells are grown in a medium containing a 1:1 mixture of Dulbecco's Modified Eagle's Medium and Ham's F12 medium with 2.5 mM L-glutamine adjusted to contain 15 mM HEPES, 0.5 mM sodium pyruvate, and 1.2 g/L sodium bicarbonate supplemented with 0.05 mM non-essential amino acids and 10% fetal bovine serum.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: M059K (ATCC® CRL-2365™)

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: brain
Disease: malignant glioblastoma; glioma
Cell Type: glial cell
Age: 33 years
Gender: male
Morphology: fibroblast
Growth Properties: adherent
DNA Profile:
Amelogenin: X,Y
CSF1PO: 10,12
D13S317: 14
D16S539: 10,12
D5S818: 11,12
D7S820: 10
THO1: 9.3
TPOX: 8
vWA: 17
Cytogenetic Analysis: Number of cells examined = 59; Modal Chromosome Number = 75 with a range of 65 to 79; Polyploidy Rate = 22%

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 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.

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 7 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 sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information), grown, and completely filled with medium at

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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 any evidence of microbial contamination. Also, check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached,** aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 flask; 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 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 10 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.
6. Incubate cultures at 37°C

Subculture Ratio: 1:6 to 1:8
Medium Renewal: Every 2 to 3 days.
Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture of Animal Cells, a manual of Basic Technique** by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

Cryopreservation Medium

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

Comments

M059K cells express normal levels of DNA-dependent protein kinase while M059J cells lack DNA-dependent protein kinase activity. M059K cells are approximately 30-fold less sensitive to ionizing radiation than M059J cells. M059K cells are less sensitive than M059J cells to the cytotoxic effects of bleomycin, N, N-bis(2-chloroethyl)-N-nitrosourea and nitrogen mustard. M059K cells are DNA double strand break repair proficient. Both cell lines are negative for glial fibrillary acidic acid (GFAP). Together these cell lines provide useful model systems in which to study the role of DNA protein kinase in cellular and molecular processes involving DNA damage recognition and repair.

References

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

Biosafety Level: 1

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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Disclaimers

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