MeT-5A (ATCC® CRL-9444™)

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 Medium 199 containing 1.5 g/L sodium bicarbonate. To make the complete growth medium, add the following components to the base medium:
- 10% fetal bovine serum (final conc.)
- 3.3 mM epidermal growth factor (EGF) (final conc.) (do not filter)
- 400 nM hydrocortisone (final conc.)
- 870 nM zinc-free bovine insulin (final conc.)
- 20 mM HEPES (final conc.)

The trace elements at the following final concentrations:
- $H_2SeO_3$: 0.3869 mg/L (Selenious acid)
- $MnO_4^{-} \times 4H_2O$: 0.0198 mg/L (Manganese chloride)
- $Na_2SiO_3 \times 9H_2O$: 14.2100 mg/L (Sodium silicate)
- $(NH_4)Fe(CN)_6 \times 4H_2O$: 0.1236 mg/L (Ammonium molybdate)
- $NH_4VO_3$: 0.0586 mg/L (Ammonium vanadate)
- $NISO_4 \times 9H_2O$: 0.0131 mg/L (Nickel sulfate)
- $SrCl_2 \times 6H_2O$: 0.0113 mg/L (Tin Chloride)


ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020 (500ml) and ATCC Catalog No. 30-2021 (100ml).

Citation of Strain
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

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. It is recommended that the cryoprotective agent be removed immediately. Centrifuge the cell suspension at approximately 125 x g for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium.
4. Transfer the vial contents to an appropriate size vessel. 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 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% 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 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. Incubate the cells at 37°C in a 5% CO₂ 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 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 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

**Subculturing Procedure**

Volumes are given for a 75 cm² flask. Increase or decrease the amount of dissociation medium needed proportionally 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 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. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 g for 5 to 10 minutes. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:4 is recommended

**Medium Renewal:** Every 2 to 3 days

**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**

The cells stain positively for vimentin, keratins and SV40 T antigen.

**References**

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

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 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
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- \( Na_2SiO_3 \times 9H_2O \) 14.2100 mg/L (Sodium silicate)
- \( (NH_4)_6Mo_7O_{24} \times 4H_2O \) 0.1236 mg/L (Ammonium molybdate)
- \( NH_4VO_3 \) 0.0585 mg/L (Ammonium vanadate)
- \( NiSO_4 \times 6H_2O \) 0.0131 mg/L (Nickel sulfate)
- \( SnCl_2 \times 2H_2O \) 0.0113 mg/L (Tin Chloride)


This medium is formulated for use with a 5% CO\(_2\) in air atmosphere.

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