



Product Sheet

## AML12 (ATCC® CRL-2254™)

Please read this FIRST



Storage Temp.  
**liquid nitrogen  
vapor phase**

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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

A 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium with 0.005 mg/ml insulin, 0.005 mg/ml transferrin, 5 ng/ml selenium, and 40 ng/ml dexamethasone, 90%; fetal bovine serum, 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: AML12 (ATCC® CRL-2254™)

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Manassas, VA 20108 USA  
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### Description

**Organism:** *Mus musculus*, mouse  
**Strain:** CD-1  
**Tissue:** liver  
**Disease:** normal  
**Cell Type:** hepatocyte  
**Age:** 3 months  
**Gender:** male  
**Morphology:** epithelial  
**Growth Properties:** adherent

### 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 submerged 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  $-130^{\circ}\text{C}$ , 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  $-70^{\circ}\text{C}$ . Storage at  $-70^{\circ}\text{C}$  will result in loss of viability.

1. Thaw the vial by gentle agitation in a  $37^{\circ}\text{C}$  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) and dispense into a  $25 \text{ cm}^2$  or a  $75 \text{ cm}^2$  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  $37^{\circ}\text{C}$  in a suitable incubator. A 5%  $\text{CO}_2$  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 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  $37^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  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



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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<sup>2</sup> flask. Incubate at 37°C in a 5% CO<sub>2</sub> in air atmosphere until cells are ready to be subcultured.



### Subculturing Procedure

Volumes used in this protocol are for 75 cm<sup>2</sup> 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 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.
6. Incubate cultures at 37°C.

**Subcultivation Ratio:** 1:4 to 1:6

**Medium Renewal:** 2 to 3 times a week.

**Note:** For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in **Culture of Animal Cells, a manual of Basic Technique** by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.



### Cryopreservation 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 AML12 (alpha mouse liver 12) cell line was established from hepatocytes from a mouse (CD1 strain, line MT42) transgenic for human TGF alpha.

By electron microscopy, these cells exhibit typical hepatocyte features such as peroxisomes and bile canalicular like structure.

AML12 cells retain the capacity to express high levels of mRNA for serum (albumin, alpha 1 antitrypsin and transferrin) and gap junction (connexins 26 and 32) proteins, and contain solely isoenzyme 5 of lactate dehydrogenase.

The cells express high levels of human TGF alpha and lower levels of mouse TGF alpha.

Expression of liver specific proteins decreases with time in culture, but is reactivated by growing the cells in serum free medium.



### References

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

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### Disclaimers

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Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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