



## Product Sheet

# TM4 (ATCC® CRL-1715™)

### 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 Ham'S F12 medium and Dulbecco's modified Eagle's medium with 1.2 g/L sodium bicarbonate and 15 mM HEPES, 92.5%; horse serum, 5%; fetal bovine serum, 2.5%

### Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: TM4 (ATCC® CRL-1715™)

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

800.638.6597 or 703.365.2700  
Fax: 703.365.2750  
Email: [Tech@atcc.org](mailto:Tech@atcc.org)

Or contact your local distributor

## Description

**Organism:** *Mus musculus*, mouse

**Tissue:** testis

**Cell Type:** Sertoli cell

**Age:** 11 to 13 days

**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 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  $-130^{\circ}\text{C}$ , 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 10 minutes.
4. Resuspend the 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
2. Incubate the flask in an upright position for several hours at  $37^{\circ}\text{C}$ . After the temperature has equilibrated, aseptically remove the contents of the flask and centrifuge at  $125 \times g$  for 5 to 10 minutes. Remove shipping medium and save for reuse. Resuspend the cell pellet in 10 mL of this medium in the original flask.
3. Incubate the culture, horizontally, at  $37^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.

## Subculturing Procedure

Volumes are given for a  $75 \text{ cm}^2$  flask. Increase or decrease the amount of dissociation medium needed



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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 which 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:** A subcultivation ratio of 1:10 to 1:50 is recommended

**Medium Renewal:** Every 3 to 4 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 TM4 cell line is reported to respond to FSH with an increase in cAMP production, but to not respond to luteinizing hormone (LH). The FSH responsiveness is much reduced compared to primary sertoli cell cultures. Constitutive plasminogen activator production is reported to be low, but is stimulated by FSH and, to a greater extent, by retinoic acid.

Tested and found negative for ectromelia virus (mousepox).



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

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