



Product Sheet

# Wt MEFs (ATCC® CRL-2991™)

Please read this **FIRST**



Storage Temp.  
**liquid nitrogen**  
vapor phase

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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: bovine calf 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: Wt MEFs (ATCC® CRL-2991™)

American Type Culture Collection  
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Manassas, VA 20108 USA  
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Or contact your local distributor

## Description

**Organism:** *Mus musculus*, mouse  
**Immortalization Method:** immortalized by transduction with SV-40 T antigen  
**Tissue:** embryo/embryo fibroblast  
**Cell Type:** fibroblast  
**Age:** embryo, 10.5-days gestation  
**Morphology:** fibroblast-like  
**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 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.

## Subculturing Procedure

Volumes used in this protocol are for  $75 \text{ cm}^2$  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  $\text{Ca}^{++}/\text{Mg}^{++}$  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 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  $37^{\circ}\text{C}$  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. Place culture vessels in incubators at  $37^{\circ}\text{C}$ .


**Subcultivation ratio:** A subcultivation ratio of 1:5 to 1:20 is recommended



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**Cryopreservation Medium**

Complete growth medium described above supplemented with 10% (v/v) DMSO.  
Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.



**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**

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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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