



Product Sheet

# ES-D3 [D3] (ATCC® CRL-11632™)

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

Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose supplemented with 0.1 mM 2-mercaptoethanol, 85%; heat-inactivated fetal bovine serum, 15%

## Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: ES-D3 [D3] (ATCC® CRL-11632™)

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  
**Strain:** 129/Sv+c/p  
**Tissue:** embryo  
**Cell Type:** pluripotent embryonic stem cell  
**Age:** embryo, blastocyst  
**Morphology:** spherical colonies  
**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.

**NOTE: Cells must be maintained on feeder layers of irradiated (12,000 rads) or mitomycin C treated (0.01 mg/ml for 90 minutes) primary mouse embryonic fibroblasts or STO cells. (see ATCC CRL-1503, STO or ATCC 56-X, irradiated STO cells).** Plate feeder layers at  $2 \times 10^6$ /T25 or  $6 \times 10^6$ /T75 one or two days **before** plating ES-D3 cells.

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 with feeder layer.
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  $125 \times g$  for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to  $25 \text{ cm}^2$  flask. Incubate at  $37^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  in air atmosphere until cells



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are ready to be subcultured.



### Subculturing Procedure

Volumes are given for a 75 cm<sup>2</sup> flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

Note: The cells can be maintained in the undifferentiated state by frequent subculture on feeder layers of mitomycin C (MITC) treated (0.01 mg/mL for 90 minutes) primary mouse embryonic fibroblasts or STO cells (see ATCC CRL-1503, STO or ATCC 56-X.2, MITC-STO cells).

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 fresh medium, aspirate and dispense onto fresh feeder layer cultures.
6. Incubate cultures at 37°C.

**Subcultivation Ratio:** A subcultivation ratio of 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 spontaneously differentiate into embryonic structures in the absence of a feeder layer or conditioned medium. They can be injected back into blastocysts and contribute to the germline. Undifferentiated cells can be genetically modified by gene targeting techniques.



### 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.

### ATCC Warranty

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### Disclaimers

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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](http://www.atcc.org)

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