



ES-D3 [D3]

CRL-3662™

Description

ES-D3 [D3] spontaneously differentiates into embryonic structures in the absence of a feeder layer or conditioned medium. The cells can be injected back into blastocysts and contribute to the germline. This product is an ATCC manufactured progeny of CRL-11632 cited in US Pat. No. 6,486,381.

Organism: *Mus musculus*, mouse

Cell Type: pluripotent embryonic stem cell

Tissue: Embryo

Morphology: spherical colonies

Growth properties: Adherent

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Storage Conditions

Product format: Frozen

Storage conditions: Vapor phase of liquid nitrogen

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C

Handling Procedures

Unpacking and 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°C, preferably in liquid nitrogen vapor, until ready for use.

Complete 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%

Handling Procedure:

To ensure 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.

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 2×10^6 /T25 or 6×10^6 /T75 one or two days **before** plating ES-D3 cells.

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. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium. and spin at approximately 125 x 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 cm² or a 75 cm² culture flask with feeder layer.

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.

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.

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

Reagents for cryopreservation: Complete growth medium supplemented with 5% (v/v) DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: ES-D3 [D3]
(ATCC CRL-3662)

References

References and other information relating to this material are available at www.atcc.org.

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