ATCC

Product Sheet

ES-D3 [D3] (ATCC® CRL-1934™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

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

Grow ES cells in Mouse ES Cell Basal Medium (ATCC SCRR-2011) that has been supplemented with the following components:
1. 0.1 mM 2-mercaptoethanol (Life Technologies Cat. No. 21985-023)
2. 1,000 U/mL mouse leukemia inhibitory factor (LIF) (Millipore Cat. No. ESG1107)
3. 10% to 15% ES-Cell Qualified FBS (ATCC® SCRR-30-2020) or an ES cell qualified serum replacement
Complete Growth Medium for Mouse ES Cells is stable for 14 days when stored at 2°C to 8°C.

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-1934™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Mus musculus*, mouse
Strain: 129S2/SvPas
Tissue: embryo
Cell Type: embryonic multipotent stem cell
Age: embryo, blastocyst
Morphology: spherical colony
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°C, preferably in liquid nitrogen vapor, until ready for use.

Handling Procedure for Frozen Cells

1. It is recommended that the 56-X.2 MITC-treated feeder cells be plated 24 hours before use at 6 x 10^6/T75 flask or 2 x 10^6/T25 flask in order to obtain a 100% confluent monolayer for stem cells growth.
2. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap above the water level. Thawing should be rapid (approximately 2 minutes).
3. 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.
4. 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.
5. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio)and dispense into a 75 cm^2 culture flask.
6. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product.

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.
Fibroblast-like feeder layer cells are present in the flasks sent by ATCC.

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°C in a 5% 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 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 original 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

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1. Plate irradiated (12,000 Rads) STO feeder layer at approximately 5.0 to 6.0 X 10^6 cells/ 75 cm^2 (confluent monolayer) at least one day before plating the ES cells.
   Note: If the colonies are close to or touching each other the culture is overgrown
2. Remove and discard culture medium.
3. 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.
4. 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.
5. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
6. Add appropriate aliquots of the cell suspension to new culture vessels pre-plated with new feeder layer.
7. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:10 is recommended
Medium Renewal: Every 2 to 3 days

Comments

The cells spontaneously differentiate into embryonic structures in the absence of a feeder layer or conditioned medium. They can be maintained in the undifferentiated state by frequent subculture (every 2 to 3 days) on confluent feeder layers (STO cells) arrested with Mitomycin-C (see ATCC 56-X.2; MITC- STO cells). Fibroblast-like feeder layer cells are present in the ampules sent by ATCC.
Note: These ES-D3 cells are not germline competent.

References

References and other information relating to this product are available online at 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

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.
While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.
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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

Additional information on this culture is available on the ATCC web site at www.atcc.org.