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™)

Description
Organism: Mus musculus, mouse
Strain: 129S2/SvPas
Tissue: Embryo
Cell Type: Pluripotent embryonic 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. Plate irradiated STO feeder layer at approximately 5.0 - 6.0 x 10^5 cells per 75 cm² at least one day before plating the ES cells.
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 xg 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 cm² culture flask.
6. 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.

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°C in a 5% CO₂ 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 xg 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 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

1. If the cells are still attached, aseptically remove approximately 5.0 - 6.0 x 10^5 cells per 75 cm² at least one day before subculturing.
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.
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Subculturing Procedure
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.
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 gentle 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

**Cryopreservation Medium**

Complete growth medium supplemented with 5% (v/v) DMSO

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

**Comments**

Undifferentiated cells can be genetically modified by gene targeting techniques. 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) treated with Mitomycin C (see ATCC 56-X.2; MITC-STO cells). These ES-D3 cells are not germline competent. Fibroblast-like feeder layer cells are present in the ampules sent by ATCC.

The cells spontaneously differentiate into embryonic structures in the absence of a feeder layer or conditioned medium.

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

**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**

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.

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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.

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Product Sheet
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