



# ES-D3 [D3]

CRL-1934™

## Description

ES-D3 [D3] is an embryonic multipotent stem cell line that was isolated from the embryo of a mouse. This cell line was deposited by T Doetschman and can be used in stem cell research.

**Cell Type:** embryonic multipotent stem cell

**Tissue:** Embryo

**Morphology:** spherical colony

**Growth properties:** Adherent

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## Storage Conditions

**Product format:** Frozen

**Storage conditions:** Vapor phase of liquid nitrogen

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## 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.

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## 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

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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.

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### Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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### Growth Conditions

**Temperature:** 37°C

**Atmosphere:** 95% Air, 5% CO<sub>2</sub>

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### 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:**

The base medium for this cell line is Mouse ES Cell Basal Medium (ATCC SCRR-2011). To make the complete medium add the following components to 500 mL base medium and mix by swirling gently:

- 1 mL (0.1 mM final concentration) 2-mercaptoethanol (Life Technologies Cat. No. 21985-023)
- 56 to 84 mL (10% to 15% final concentration) ES-Cell Qualified FBS (ATCC SCRR-30-2020)
- 1,000 U/mL mouse leukemia inhibitory factor (LIF) (Millipore Cat. No. ESG1107).  
\*NOTE: LIF can be omitted from the culture media as long as 56-X.2 (MITC-treated STO) is used as a feeder layer since these cells produce LIF.

Complete Growth Medium for Mouse ES Cells is stable for 14 days when stored at 2°C to 8°C.

**Handling Procedure:**

**Note:** To maintain the cells in the undifferentiated state they must be grown on confluent feeder layers of irradiated STO cells (see 56-X, irradiated STO cells).

1. It is recommended that the 56-X.2 MITC-treated feeder cells be plated 24 hours before use at  $6 \times 10^6$ /T75 flask or  $2 \times 10^6$ /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<sup>2</sup> culture flask.
6. Incubate the culture at 37°C in a suitable incubator. A 5% CO<sub>2</sub> in air atmosphere is recommended if using the medium described on this product.

**Subculturing procedure:****Feeder Layer Preparation**

56-X.2 cells should be seeded one day prior to use.

The medium to use when initiating the feeder layer is DMEM with 10% FBS. This medium is prepared by aseptically combining:

56 mL FBS (ATCC 30-2020)

500 mL DMEM (ATCC 30-2002)

1. Thaw the frozen feeder cell vial(s) per the ATCC product sheet. Wipe, spray, and/or soak the ampoule(s) with 70% ethanol (or equivalent disinfectant) and allow the ampoule(s) to dry.
2. Aseptically open the ampoule(s). Withdraw cells and transfer to a sterile 15 mL centrifuge tube. If more than one ampoule was thawed, the contents may be pooled into a single centrifuge tube.
3. Slowly add pre warmed feeder layer medium to the centrifuge tube by running  $10 \pm 2$  mL down the side. Centrifuge the tube at  $275 \pm 125 \times g$  for  $10 \pm 2$  minutes. Aseptically remove and discard the supernatant from the centrifuge tube.
4. Resuspend the cell pellet with feeder layer medium so that a final volume of 10 mL is achieved. Count the resuspended cells. Calculate the volumes of cell suspension and feeder layer medium needed to plate the feeder. Aseptically transfer the calculated volumes of cell suspension and feeder layer medium to appropriate vessel(s).
5. Incubate the culture in a CO<sub>2</sub> incubator set to  $5\% \pm 1\%$  CO<sub>2</sub> and 35.0 to 37.0 °C until ready for use.
6. Plate irradiated (12,000 Rads) STO feeder layer at approximately  $8.0 \times 10^4$  viable cells/ cm<sup>2</sup> at least one day before plating the ES cells. After one day of incubation the vessel(s) are ready for use in CRL-1934 cultures.

### **Initiation of CRL-1934 Cell Culture**

1. Thaw a vial of CRL-1934 cells per the ATCC product sheet - Handling Procedure for Frozen Cells. Wipe, spray, and/or soak the ampoule(s) with 70% ethanol (or equivalent disinfectant) and allow the ampoule(s) to dry.
2. Aseptically open the ampoule(s). Withdraw cells and transfer to a sterile 15 mL centrifuge tube.
3. Slowly add pre-warmed complete growth medium to the centrifuge tube by running  $12 \pm 2$  mL down the side. Centrifuge the tube at  $275 \pm 125 \times g$  for  $10 \pm$

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- 2 minutes. Aseptically remove and discard the supernatant from the centrifuge tube.
4. Resuspend the cell pellet with 10 mL of culture medium. Aseptically transfer the contents of the centrifuge tube to a T75 flask containing the prepared 56-X.2 feeder layer. Add sufficient culture medium to the flask to bring the final volume to 15mL. NOTE: Remove the feeder layer media from the flask before adding the CRL-1934 cell suspension.
  5. Incubate the culture in a CO<sub>2</sub> incubator set to 5% ± 1% CO<sub>2</sub> and 35.0 to 37.0 °C. Observe and examine the culture every 1-2 days. If a fluid renewal/addition is needed, perform the fluid renewal/addition. Aseptically remove the culture medium from the flask and discard. Add an equivalent volume of fresh culture medium to the flask. Alternatively, perform a fluid addition by adding fresh culture medium to the flask without removing the existing medium. Return the culture to the incubator after fluid renewal/addition.

**Subculture** before the CRL-1934 colonies are close to or touching each other. The CRL-1934 cells should never become 100% confluent (although the 56-X.2 feeder cells may be 100% confluent). Attached cells are subcultured using 0.25% Trypsin 0.53 mM EDTA (ATCC 30-2101). The action of the 0.25% Trypsin – 0.53mM EDTA (ATCC 30-2101) is halted by adding culture medium to the detached cells. A split ratio of 1:3 to 1:10 is used when subculturing.

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

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### 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-1934)

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### References

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

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