



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.

Organism: *Mus musculus*, mouse

Cell Type: embryonic multipotent stem cell

Tissue: Embryo

Morphology: spherical colony

Growth properties: Adherent

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

Atmosphere: 95% Air, 5% CO₂

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 6×10^6 /T75 flask or 2×10^6 /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 $125 \times g$ 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 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.

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 ± 2 mL down the side. Centrifuge the tube at $275 \pm 125 \times g$ for 10 ± 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 CO₂ incubator set to $5\% \pm 1\%$ CO₂ and 35.0 to 37.0 °C until ready for use.
6. Plate irradiated (12,000 Rads) STO feeder layer at approximately 8.0×10^4 viable cells/ cm² 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 ± 2 mL down the side. Centrifuge the tube at $275 \pm 125 \times g$ for 10 ± 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 CO₂ incubator set to $5\% \pm 1\%$ CO₂ 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)

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)

References

References and other information relating to this material are available at

www.atcc.org.

Warranty

The product is provided 'AS IS' and the viability of ATCC® products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet, website, and Certificate of Analysis. For living cultures, ATCC lists the media formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory results, a change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP standards, typicality, safety, accuracy, and/or noninfringement.

Disclaimers

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. Any proposed commercial use is prohibited without a license from ATCC.

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 or complete and the customer bears the sole responsibility of confirming the accuracy and completeness of any such information.

This product is sent on the condition that the customer is responsible for and

assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, and use of the ATCC product including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. As a condition of receiving the material, the customer agrees that any activity undertaken with the ATCC product and any progeny or modifications will be conducted in compliance with all applicable laws, regulations, and guidelines. This product is provided 'AS IS' with no representations or warranties whatsoever except as expressly set forth herein and in no event shall ATCC, its parents, subsidiaries, directors, officers, agents, employees, assigns, successors, and affiliates be liable for indirect, special, incidental, or consequential damages of any kind in connection with or arising out of the customer's use of the product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

Please see the material transfer agreement (MTA) for further details regarding the use of this product. The MTA is available at www.atcc.org.

Copyright and Trademark Information

© ATCC 2023. All rights reserved.

ATCC is a registered trademark of the American Type Culture Collection.

Revision

This information on this document was last updated on 2022-12-12

Contact Information

ATCC

10801 University Boulevard

Manassas, VA 20110-2209

USA

US telephone: 800-638-6597

ES-D3 [D3]

CRL-1934

Product Sheet

Worldwide telephone: +1-703-365-2700

Email: tech@atcc.org or contact your local distributor
