ESF 158 (ATCC® SCRC-1016™)

Please read this FIRST

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: ESF 158 (ATCC® SCRC-1016™)

Description

Organism: Mus musculus, mouse
Tissue: inner cell mass
Cell Type: embryonic stem cell
Age: embryo
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

Complete Medium for Feeder Cells
Feeder cells may be grown in medium containing fewer growth factors than those required by the ES cells. Feeder cells are available from ATCC. Consult the product sheet provided for the feeder cells you wish to use for medium requirements.

Feeder cells should be initiated 24-48 hours prior to inoculating with embryonic stem (ES) cells.

Feeder Cells
ATCC recommends culturing EDJ 22 on mouse embryonic fibroblasts (MEFs) that have been mitotically arrested by either irradiation or treatment with Mitomycin-C. EDJ 22 cells have been cultured on mitotically arrested EDJ 22 cells. It is best to use feeder cells within 24-48 hours of initiation.

Embryonic Stem (ES) Cells

1. 30 Minutes Prior to Handling Cells – Pre-warm complete growth medium for ES cells at 37°C for at least 30 minutes before adding to cells.
2. One Hour Prior to Thawing the ES Cells – Perform a 100% medium change for the MEFs using complete growth medium for ES cells.
3. Thaw the vial of ES cells 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 90 seconds).
4. Remove the vial from the water bath before the contents are completely 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.
5. Transfer the vial’s contents plus 5 mL of complete growth medium for ES cells to a 15 mL centrifuge tube. Use an additional 1 mL of media to rinse the vial and transfer the liquid to the 15 mL tube. Add 4 mL of complete growth medium for ES cells to bring the total volume to 10 mL.
6. Spin the cells at 270 x g for 5 min. Aspirate the supernatant and resuspend the pellet in 2 mL of complete growth medium for ES cells.
7. Add the 2 mL of cell suspension to the appropriate size flask containing feeder cells and fresh
Incubate the culture at 37°C in a humidified 5% CO2/95% air incubator.

Add 3.0 mL of 0.25% (w/v) Trypsin / 0.53 mM EDTA solution (ATCC® 30-2101) and place in incubator.

Resuspend in enough complete growth medium for ES cells to reseed new vessels at the desired split.

Aspirate the medium from the flask(s) containing ES cells.

Daily maintain a sufficient number of flasks that have been pre-plated with MEFs in complete medium.

Dislodge the cells by gently tapping the side of the flask then wash the cells off with 7-10 mL of fresh Complete Growth Medium for ES Cells.

Biosafety Level: 1

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Product Sheet
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Cryopreservation Medium

To freeze ES cells:
1. Follow steps 2 - 6 from the Subculturing Procedure above.
2. Resuspend the pellet in complete growth medium. Add approximately 5 mL for each T225.
3. Perform cell count and dilute the cell suspension to 6 x 10^6 cells / mL.
4. Prepare 2X Freezing Media that contains 60% complete growth medium for ES cells, 20% DMSO, and 20% FBS. Place at 4°C.
5. Add an equal volume of cold 2X Freezing Media to the cell suspension. The final concentration should be 3 x 10^6/mL.
6. Aliquot 1 mL of the cell suspension into each cryovial (3 x 10^6 vial).
7. Place the vials into a styrofoam-insulated container and place in a -80°C freezer overnight. The next day transfer the vials into liquid nitrogen.

Cryoprotectant Medium: Complete growth medium supplemented with an additional 10% FBS and 10% DMSO.

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

Comments

The clonal embryonic stem cell line #693 ES ESF 158 was derived from a strain ESF 158 mouse blastocyst [PubMed: 11730008]. The ES cells were shown to populate the germ line of two host blastocyst donors, FVB/NJ (FVB) and the coisogenic strain C57BL/6-Tyrc-2J (c2J). Coat-color chimera production was high using c2J blastocysts while FVB blastocysts produced a low number of chimeras [PubMed: 11730008].

References

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

Citation of Strain

American Type Culture Collection
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Or contact your local distributor

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.

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Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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