MEF (C57BL/6) [MEF-BL/6-1]

SCRC-1008™

Description

MEF (C57BL/6) [MEF-BL/6-1] is a fibroblast cell that was isolated from the stroma of a mouse. This cell can be used as a feeder layer to support the growth of embryonic stem (ES) cells and for the maintenance of ES cells in the undifferentiated state. The growth of these cells must be arrested before they can be used as a feeder layer. ATCC has successfully treated the cells with mitomycin C for use as a feeder layer. If the MEFs are being used as a feeder layer for ES cells, it is not recommended to use them past passage no. 6 (P6).

Organism: *Mus musculus*, mouse
Cell Type: fibroblast
Tissue: Embryo
Age: 14 days gestation
Gender: Male and female mixed
Morphology: Fibroblast
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% CO_2

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 ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium:
fetal bovine serum to a final concentration of 15%

This medium is formulated for use with a 5% CO2 in air atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO2 in air atmosphere is then recommended).

Handling Procedure: To insure the highest level of viability, be sure to warm media to 37°C before using it on the cells.
Flasks do not need to be coated before plating MEFs.

1. Thaw the vial 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.
2. Remove the vial from the water bath as soon as the contents are half way thawed (approximately 90 seconds), 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.
3. Transfer the vial's contents plus 5 mL of completes DMEM 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 DMEM to bring the total volume to 10 mL.
4. Gently mix and pellet the cells by centrifugation at 270 x g for 5 minutes.
5. Discard the supernatant and resuspend the cells with 10 mL fresh growth medium (warm) and plate the cells at seed density of $1X10^4$ cells/cm^2.
6. Add fresh growth medium (warm) to the appropriate size flask.
7. Incubate 37°C in a 5%CO_2 in air atmosphere.
8. Fluid change twice a week or when pH decreases.

It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).

Subculturing procedure: To insure the highest level of viability, be sure to warm

media and Trypsin / EDTA to 37ºC before using it on the cells. Cells should be split when they reach confluency. A split base on seed density of 2 X 10^4 cells/cm^2 is recommended.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 1XPBS (SCRR-2201) solution to remove all traces of serum, which contain trypsin inhibitor.
3. Add Trypsin-EDTA (0.25% Trypsin-0.53 mM EDTA solution, ATCC# 30-2101) solution to the flask (Table 1) and incubate for 2 minutes. Gently tapping the flask, observe cells under an inverted microscope. Cells usually detach in 2 to 3 minutes.
4. Add an equal volume complete of the growth medium (Table 1) and rinse surface of the flask to detach all the cells. Gently pipetting up and down will break cell clumps.
5. Transfer all cells into a centrifuge bottle or tube and centrifuge at 270 x g for 5 minutes.
6. Remove and discard the supernatant
7. Add 10 mL complete growth medium to the cell pellet and with 10 mL pipette resuspend the cells gently (create a single-cell suspension).
8. Add more complete growth medium (Table 1) to the cell suspension as needed to plate cells at approximately 0.8 X 10^4 cells/cm^2.
9. Place flasks in the incubator @ 37°C with a 5% CO_2 in air atmosphere

Flask/Plate	Growth Area (cm^2)	1xPBS (mL)	Trypsin/EDTA (mL)	Equal vol. Complete Growth Medium (mL)	Growth Medium (mL)
T225	225	10 ± 0.2	6 ± 0.2	6 ± 0.2	30
75	75	5 ± 0.1	3 ± 0.1	3 ± 0. 1	12
T25	25	3 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	6
6 well	9.5	1 ± 0.1	1 ± 0.1	1 ± 0.1	3

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in *Culture of Animal Cells, a manual of Basic Technique* by R. Ian

Freshney, 5th edition, published by Alan R. Liss, N.Y., 2005.

Subcultivation Ratio: 1:5 to 1:8

Reagents for cryopreservation: Complete growth medium supplemented with 40% (v/v) FBS and 10% (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: MEF (C57BL/6) [MEF-BL/6-1] (ATCC SCRC-1008)

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

Revision

This information on this document was last updated on 2023-04-16

Contact Information

ATCC
10801 University Boulevard
Manassas, VA 20110-2209
USA
US telephone: 800-638-6597
Worldwide telephone: +1-703-365-2700
Email: tech@atcc.org or contact your local distributor