MEF (C57BL/6) [MEF-BL/6-1] (ATCC® SCRC-1008™)

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
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).

Citation of Strain
If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: MEF (C57BL/6) [MEF-BL/6-1] (ATCC® SCRC-1008™)

Handling Procedure for Frozen Cells
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 complete 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 1X10^6 cells/cm^2.
6. Add fresh growth medium (warm) to the appropriate size flask.
7. Incubate 37°C in a 5%CO2 in air atmosphere.
8. Fluid change twice a week or when pH decreases.

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.

Subculturing Procedure
To insure the highest level of viability, be sure to warm media and Trypsin / EDTA to 37°C 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

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

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.

### Cryopreservation Medium

Complete growth medium, supplemented with an additional 40% FBS and 10% DMSO(v/v)

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

### 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.

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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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