**Product Sheet**

**MEF (DR4) (ATCC® SCRC-1045™)**

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**Please read this FIRST**

- **Storage Temp.** liquid nitrogen vapor phase
- **Biosafety Level** 1

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**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 (DR4) (ATCC® SCRC-1045™)

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**Description**

**Organism:** *Mus musculus*, mouse  
**Strain:** DR4  
**Tissue:** Embryo  
**Cell Type:** Fibroblast  
**Age:** 14 days gestation embryo  
**Gender:** Male and female mixed  
**Morphology:** fibroblast  
**Growth Properties:** Adherent

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**Batch-Specific Information**

Refer to the Certificate of Analysis for batch-specific test results.

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**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.

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**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.

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**Handling Procedure for Frozen Cells**

It is not necessary to coat flasks with gelatin prior to plating cells, if tissue culture quality flasks are used. To insure the highest level of viability, be sure to warm media to 37°C before using it on the cells. It is recommended to seed cells at 1.2 X 10⁶ cells/cm² post-thaw.

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 growth medium 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 complete growth medium (warm) and count cells.
6. If necessary, add more fresh complete growth medium (warm) to obtain a seeding density of 1.2 X 10⁶ cells/cm². Transfer appropriate volumes of cell suspension to culture vessels.
7. Add more complete growth medium to obtain the total volume recommended for the culture vessels seeded.
8. Incubate 37°C in a 5% CO₂ in air atmosphere.
9. Fluid change twice a week or when pH decreases.

**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 based on seeding density of 6 X 10³ cells/cm² is recommended.

**Note:** Volumes used in this protocol are for 75cm² flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 5.0 mL 1XPBS (ATCC Catalog No. SCRR-2201) solution to remove all traces of serum, which contain trypsin inhibitor.
Add 3.0 mL 0.25% Trypsin-0.53 mM EDTA solution (ATCC Catalog No. 30-2101) solution to the flask and incubate for 2 minutes. Gently tap the flask and observe cells under an inverted microscope. Cells usually detach in 1 to 2 minutes.

Add 3.0 mL complete growth medium and rinse the surface of the flask to detach all the cells. Gently pipette up and down will break cell clumps.

Transfer all cell suspension into a centrifuge tube and centrifuge at 270 xg for 5 minutes.

Remove and discard the supernatant.

Transfer all cell suspension into a centrifuge tube and centrifuge at 270 xg for 5 minutes.

Adjust volume as needed to seed vessels at approximately 6 X10^6 cells/cm².

Place flasks in incubator at 37 °C with 5% CO₂ in air atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO₂ in air atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO₂ in air atmosphere.


Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:7 is recommended

Medium Renewal: Twice a week or when pH decreases

Comments

The growth of these cells should be arrested before being used as a feeder layer. ATCC has successfully irradiated and 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. 7 (PT).

ATCC tested that this cell line is resistant to:
- G 418 (neomycin): 200 microgm/mL
- Puromycin: 0.4 microgm/mL
- Hygromycin: 110 microgm/mL
- 6-Thioguanine: 2.5 microgm/mL

References

References and other information relating to this product are available online at 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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Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

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