



# MEF (DR4)

SCRC-1045™

## Description

MEF (DR4) is a fibroblast cell that was established from embryonic day 14 (E14) DR4 mouse embryos obtained from The Jackson Laboratory. The cells can be used as a feeder layer to support the growth of engineered embryonic stem (ES) cells with multiple drug selections and for the maintenance of ES cells in the undifferentiated state.

**Organism:** *Mus musculus*, mouse

**Cell Type:** fibroblast

**Tissue:** Embryo

**Age:** 14 days gestation

**Gender:** Male and female mixed

**Morphology:** fibroblast

**Growth properties:** Adherent

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## Storage Conditions

**Product format:** Frozen

**Storage conditions:** Vapor phase of liquid nitrogen

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## 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.

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## 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.

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## Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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## Growth Conditions

**Temperature:** 37°C

**Atmosphere:** 95% Air, 5% CO<sub>2</sub>

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## 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% CO<sub>2</sub> in air atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO<sub>2</sub> in air atmosphere is then recommended).

**Handling Procedure:** 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  $1.2 \times 10^4$  cells/cm<sup>2</sup> 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 10mL fresh complete growth medium (warm) and count cells.
6. If necessary, add more fresh complete growth medium (warm) to obtain a seeding density of  $1.2 \times 10^4$  cells/cm<sup>2</sup>. 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% CO<sub>2</sub> in air atmosphere.
9. 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 37°C before using it on the cells. Cells should be split when they reach confluency. A split based on seeding density of  $6 \times 10^3$  cells/cm<sup>2</sup> is recommended.

**Note:** Volumes used in this protocol are for 75cm<sup>2</sup> 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.
3. 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.
4. 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.
5. Transfer all cell suspension into a centrifuge tube and centrifuge at 270 xg for 5 minutes.
6. Remove and discard the supernatant.
7. Add complete growth medium to the cell pellet and with 10 mL pipette gently resuspend the cells gently to create a single-cell suspension.
8. Adjust volume as needed to seed vessels at approximately  $6 \times 10^3$  cells/cm<sup>2</sup>.
9. Place flasks in incubator at 37° with 5% CO<sub>2</sub> in air atmosphere.

**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 Wiley-Liss, N.Y., 2005.

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

**Medium Renewal:** Twice a week or when pH decreases

**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 (DR4) (ATCC SCRC-1045)

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## References

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

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