

## SCRC-1040.2a<sup>™</sup>

## Description

MEF (CF-1) MITC is a fibroblast cell that was isolated from an embryo of a mouse. The cells can be used as a feeder layer.

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<sub>1</sub>

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



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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<sub>2</sub>

## 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

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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, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at  $-70^{\circ}$ C. Storage at  $-70^{\circ}$ C will result in loss of viability.

To insure the highest level of viability, be sure to warm media to 37°C before use.

Note: 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 contents to a 15 mL centrifuge tube containing 5 mL of complete growth medium. Use an additional 1 ml of complete growth media to rinse the vial and transfer it to the 15 mL tube. Add 4 mL more complete growth medium to bring the total volume to 10 mL.
- 4. Gently mix and pellet the cells by centrifugation at 270 xg for 5 minutes.
- 5. Discard the supernatant and resuspend the cells with fresh complete growth medium (warm) and plate the cells at a seeding density of 6 X 10<sup>4</sup> cells/cm<sup>2</sup>.
- 6. Add fresh complete growth medium (warm) to the appropriate size flask.
- 7. Place flasks in a 5% CO<sub>2</sub> incubator at 37°C.
- 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

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reach its normal pH (7.0 to 7.6).

Note: Cells should be plated 24 hours before use as a feeder layer for ES cells and kept for no more than 7 days.

#### **Subculturing procedure:**

Once the feeder cells have attached, the culture medium can be changed to accommodate the cells to be supported. It is recommended that the feeder cells be plated 24 hours before use at 6  $\times$  10<sup>4</sup> cells/cm<sup>2</sup> in order to obtain a supportive monolayer for stem cell growth.

Medium Renewal: Twice a week or when pH decreases

#### Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: MEF (CF-1) MITC (ATCC SCRC-1040.2a)

#### References

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

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### Revision

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