# Mouse Embryonic Fibroblasts (MEF)

Catalog Number: M7540-57

## Cell Specification

Mouse Embryonic Fibroblasts (MEF) are used to support the growth of undifferentiated mouse or human ES and iPS cells [1]. They provide both a substrate for the ES cells to grow on and secrete many factors necessary for ES cells to maintain pluripotency. MEF cells are isolated from mouse embryos and are used at their early passages [2]. Before MEF cells are used as feeder cells, they must be treated by irradiation or mitomycin C to stop the cells from further dividing.

MEF from ScienCell Research Laboratories are isolated from day 13 mouse embryos. MEF are cryopreserved at primary culture and delivered frozen. Each vial contains 5 x $10^6$ cells in 1 ml volume. MEF are characterized by immunofluorescent imaging using antibodies to fibronectin. MEF are negative for mycoplasma, bacteria, yeast and fungi. MEF are guaranteed to further culture over 5 population doublings at the conditions provided by ScienCell Research Laboratories.

## Recommended Medium

It is recommended to use DMEM F12 (cat. no. 09411) 10% FBS (Cat. No. 0010, 0025 or 0500) for the culturing of MEF cells *in vitro*.

## Product Use

MEF are used as feeder layer in mouse and human ES or iPS cell culture. They are for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

## Storage

Directly and immediately transfer cells from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture is needed for experiments.

## Shipping

Dry ice.

### Reference

[1] BRADLEY, A. (1987). Production and analysis of chimaeras. In *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach,* (ed. E. J. Robertson), pp. 113-151. Oxford: IRL Press.
[2] Nagy et al. (2006) Preparing Mouse Embryo Fibroblasts Cold Spring Harbor Protocols. 2006: pdb.prot 4398.

# Instruction for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37ºC waterbath and return them to culture as quickly as possible with minimal handling!

**Set up culture after receiving the order:**

1. Prepare sterile 0.1% gelatin coated dishes (10cm dishes are recommended). Add 3mL of cooled 0.1% gelatin to a dish and leave in 37ºC incubator for 1 hour.
2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically make 10% fetal bovine serum (cat. no. 0010, 0025 or 0500) in DMEM F12 (cat. no. 09411).
3. Completely aspirate gelatin from coated dishes. No need to rinse dishes and add 5mL complete medium. Leave dishes in the hood and go to thaw the MEF.
4. Place the vial in a 37ºC waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, poly-lysine coated culture vessels. A seeding density of 50,000 cells/$cm^2$ is recommended.
   *Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that cells are plated in gelatin coated culture vessels to promote MEF cell attachment.*
6. Gently rock the vessels to distribute cells evenly.
7. Return the culture vessels to the incubator.
8. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter.

**Maintenance of Culture:**

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.
2. Change the medium every other day thereafter, until the culture is approximately 50% confluent.

3. Once the culture reaches 50% confluence, change medium every day until the culture is ready for subculture.

**Subculture:**

1. Subculture the cells when they are over 90% confluent.
2. Prepare 0.1% gelatin coated dishes.
3. Warm medium, trypsin/EDTA solution (T/E, cat. no. 0103), trypsin neutralization solution (TNS, cat. no. 0113), and DPBS (cat. no. 0303) to **room temperature**. We do not recommend warming the reagents and medium at 37ºC waterbath prior to use.
4. Rinse the cells with DPBS.
5. Add 10 ml of DPBS first and then 2 ml of trypsin/EDTA solution into dish (in the case of 10cm dish); gently rock the dish to make sure cells are covered by trypsin/EDTA solution; incubate the dish at 37ºC incubator for 1 to 2 minutes or until cells are completely rounded up (monitored with inverted microscope). During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, cat. no. 0500); transfer trypsin/EDTA solution from the dish to the 50 ml centrifuge tube (a few percent of cells may detached); continue incubate the dish at 37ºC for 1 minutes (no solution in the dish at this moment); at the end of trypsinization, one hand hold one side of dish and the other hand gently tap the other side of the dish to detach cells from attachment; check the dish under inverted microscope to make sure all cells are detached, add 5 ml of trypsin neutralization solution to the dish and transfer detached cells to the 50 ml centrifuge tube; add another 5 ml of TNS to harvest the residue cells and transfer it to the 50 ml centrifuge tube. Examine the dish under inverted microscope to make sure the cell harvesting is successful by looking at the number of cells left behind. There should be less than 5%.
   *Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.*
6. Centrifuge the 50 ml centrifuge tube (harvested cell suspension) at 1000 rpm (*Beckman Coulter* Allegra 6R centrifuge or similar) for 5 min; re-suspend cells in growth medium.
7. Count cells and plate cells in a new, gelatin coated dish with cell density as recommended.

*Caution: Handling animal derived products is potentially biohazardous. Proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of animal origin as the minimum precaution against contamination [1].*

[1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. J Tissue Culture Methods. 11(4).