Mouse Embryonic Fibroblasts (MEF)

Catalog Number: M7540

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 immunofluorescentr method with 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 with 10% FBS for the culturing of MEF *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 37^{o}C waterbath and return them to culture as quickly as possible with minimal handling!

Set up culture after receiving the order:

1. Prepare a poly-lysine coated flask (2 μg/cm^2, T-75 flask is recommended). Add 10 ml of sterile cell culture-grade water to a T-75 flask and then add 150 μl of ply-lysine stock solution (1 mg/ml, ScienCell cat. no. 0403). Leave the flask in incubator overnight (minimum one hour at 37^{o}C incubator).
2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.
3. Aspirate poly-lysine solution and rinse the flask with sterile cell culture-grade water twice. Add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.
4. Place the vial in a 37^{o}C 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 10,000 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 poly-lysine coated culture vessels that promote mesenchymal stem cell attachment.
6. Replace the cap or cover of flask, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange.
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. A healthy culture will display fibroblast/smooth muscle cell-like morphology, usually in a scattered single cells rather than a homogeneous bundle or sheet of cells; and the cell number will be doubled after two to three days in culture.

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 poly-lysine coated cell culture flasks (2 $\mu g/cm^2$).
3. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to **room temperature**. We do not recommend warming the reagents and medium at 37^oC waterbath prior to use.
4. Rinse the cells with DPBS.
5. Incubate cells with 10 ml of trypsin/EDTA solution (in the case of T-75 flask) until 80% of cells are rounded up (monitored with microscope). Add 10 ml of trypsin neutralization solution to the digestion immediately and gently rock the culture vessel.
   Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.
6. Harvest and transfer released cells into a 50 ml centrifuge tube. Rinse the flask with another 10 ml of growth medium to collect the residue cells. Examine the flask under microscope to make sure the harvesting is successful by looking at the number of cells left behind. There should be less than 5%.
7. Centrifuge the harvested cell suspension at 1000 rpm for 5 min and resuspend cells in growth medium.
8. Count cells and plate them in a new, poly-lysine coated flask with cell density as recommended.

Caution: Handling animal derived products is potentially bioharzadous. Proper precautions mush 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 human 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).