Mouse Embryonic Fibroblasts (MEF)

Cat. No. M7570-5-mt

Cell Specification

Mouse Embryonic Fibroblasts (MEF) are used to support the growth of mouse and human pluripotent stem cells [1]. MEF not only provide a substrate for pluripotent stem cells to grow on, but also secrete critical growth factors to maintain stem cell pluripotency. MEF are isolated from mouse embryos and used at early passages [2]. To serve as feeder cells, MEF must be treated with mitomycin C or by irradiation to prevent cell proliferation. The treated cells can also be used to generate conditioned medium for feeder-free culture of pluripotent stem cells.

MEF-mt (Cat. No. M7570-5-mt) from ScienCell Research Laboratories are isolated from E13 embryos of CF1 mice and have been treated with mitomycin C to prevent further cell division. They are cryopreserved at passage 3 and delivered frozen. Each vial contains 5 x 10^6 cells in 1 ml volume. MEF-mt are characterized by immunofluorescence with antibodies specific to fibronectin. MEF-mt are negative for mycoplasma, bacteria, yeast and fungi.

Recommended Medium

It is recommended to use DMEM (Cat. No. 09221) containing 10% fetal bovine serum (FBS, Cat. No. 0010, 0025, 0500) for the culturing of MEF-mt cells *in vitro*.

Product Use

MEF-mt are used as feeder layer in mouse and human pluripotent stem cell culture. They are for research use only. They are 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.

References

[1] Bradley A. (1987) "Production and analysis of chimaeras." In Robertson EJ, *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach* (pp 113-51). Oxford: IRL Press.

[2] Nagy A, Gertsenstein M, Vintersten K, Behringer R. (2006) "Preparing Mouse Embryo Fibroblasts." *Cold Spring Harbor Protocols.* pdb.prot 4398.

Instruction for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37^{o}C water bath and return the cells to culture as quickly as possible with minimal handling!

Initiating the culture:

1. Prepare 0.2% gelatin (Cat. No. 0423) coated culture vessel. Use enough volume of gelatin to cover the entire culture surface. Leave the vessel in 37^{o}C incubator for 1 hour.
2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer 10% fetal bovine serum (FBS, Cat. No. 0010, 0025, 0500) to DMEM medium (Cat. No. 09221) with a pipette.
3. Completely aspirate 0.2% gelatin from the coated vessel. No need to rinse the vessel and add complete medium to cover the culture surface. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Place the frozen vial in a 37^{o}C water bath. Hold and rotate the vial gently until the contents completely thaw. Remove the vial from the water bath promptly, wipe it down with 70% ethanol and transfer it to the sterile field.
5. Remove the cap carefully without touching the interior threads. Gently resuspend and dispense the contents of the vial into a 15 ml conical centrifuge tube. Add appropriate volume of complete medium to the cells, mix well gently and plate cells into the coated vessel at the required density.
   Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture. It is also important that cells are plated in gelatin coated culture vessels to promote cell attachment.
6. Replace the cap or lid, and gently rock the vessel to distribute the cells evenly.
7. Return the culture vessel to the incubator.
8. For the best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter.

Maintaining the culture:

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.

2. Change the medium every other day thereafter. Or cells can be used as feeders for human and mouse pluripotent stem cell culture.

MEF-mt cells have been treated with mitomycin C to prevent cell division.

Caution: Handling animal derived products is potentially biohazardous. Always wear gloves and safety glasses when working with 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 WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Culture Methods*. 11: 191-9.