Mouse Embryonic Fibroblasts-Mitomycin C treated from CD-1 mouse (MEF-mt)

Catalog #M7540-2-mt (old Cat. #M7550-2)

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 from ScienCell Research Laboratories are isolated from embryonic day 14 CD-1 mouse embryos. These cells have been treated with mitomycin C to prevent further cell division. They are cryopreserved at passage 3 and delivered frozen. Each vial contains 2 x 10^6 cells in 1 ml volume. MEF-mt are characterized by immunofluorescence with antibody specific to fibronectin. MEF-mt are negative for mycoplasma, bacteria, yeast, and fungi. MEF are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories; *however, MEF-mt are not recommended for expanding as mitomycin C treatment prevents further cell proliferation*.

Recommended Medium

It is recommended to use DMEM (Cat. #09221) supplemented with 10% fetal bovine serum (FBS, Cat. #0010, 0025, 0500) for culturing MEF-mt *in vitro*.

Product Use

MEF-mt are used as a 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

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are 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.

Instructions for culturing primary cells

Caution: Cryopreserved primary cells are very delicate. Thaw the vial in a 37ºC water bath and return the cells to culture as quickly as possible with minimal handling! Do not centrifuge the cells after thawing as this can damage the cells.

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37ºC, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Prepare a poly-L-lysine-coated culture vessel (2 µg/cm^2, T-75 flask is recommended). To obtain a 2 µg/cm^2 poly-L-lysine-coated culture vessel, add 10 ml of sterile water to a T-75 flask and then add 15 µl of poly-L-lysine stock solution (10 mg/ml, Cat. #0413). Leave the vessel in a 37ºC incubator overnight (or for a minimum of one 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 supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.
3. Rinse the poly-L-lysine-coated vessel twice with sterile water and then add 20 ml of complete medium. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Place the frozen vial in a 37ºC water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.
5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, poly-L-lysine-coated culture vessel.

   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 poly-L-lysine-coated culture vessels to promote cell attachment.
6. Replace the cap or lid of the culture vessel and gently rock the vessel to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.
7. Return the culture vessel to the incubator.
8. Do not disturb the culture for at least 16 hours after initiation. Refresh culture medium the next day to remove residual DMSO and unattached cells.
9. Cells are then ready for experiments as a feeder layer.

MEF-mt are not recommended to be subcultured as mitomycin C treatment prevents further cell proliferation.

Note: We do not recommend cryopreservation of primary cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee primary cells cryopreserved by the end user.

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.