Mouse Embryonic Fibroblasts from CF1 mouse (MEF)

Catalog #M7570

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 from ScienCell Research Laboratories are isolated from embryonic day 13 CF1 mouse embryos. These cells are cryopreserved at P0 and delivered frozen. Each vial contains 1 x 10^6 cells in 1 ml volume. MEF are characterized by immunofluorescence with antibody specific to fibronectin. MEF are negative for mycoplasma, bacteria, yeast, and fungi. MEF are guaranteed to further expand for 5 population doublings under the conditions provided by ScienCell Research Laboratories.

Recommended Medium

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

Product Use

MEF 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 cells

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

Initiating the culture:

1. Prepare 0.1% gelatin (Cat. #0423) coated culture vessel. Use enough volume of gelatin to cover the entire culture surface. Leave the vessel in a 37°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% FBS (Cat. #0010, 0025 or 0500) to DMEM (Cat. #09221).
3. Completely aspirate gelatin from the coated vessel. It is not necessary to rinse the vessel. 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°C 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, gelatin-coated culture vessel. A seeding density of 5,000 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 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 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. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells.

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, until the culture is approximately 70% confluent.
3. Once the culture reaches 70% confluence, change medium every day until the culture is ready for subculture.

Subculturing:

1. Subculture when the culture reaches 90% confluency or above.
2. Prepare 0.1% gelatin-coated culture vessels as described before.
3. Warm complete medium, trypsin/EDTA solution (T/E, Cat. #0103), T/E neutralization solution (TNS, Cat. #0113), and DPBS (Ca^{++}- and Mg^{++} -free, Cat. #0303) to **room temperature**. We do not recommend warming reagents and medium in a 37ºC water bath prior to use.
4. Rinse the cells with DPBS.
5. Dilute 2 ml of T/E solution with 10 ml of DPBS and mix well. Apply the diluted T/E solution to the cells. Gently rock the vessel to ensure complete coverage of cells by T/E solution. Incubate the flask in a 37ºC incubator for 1 to 2 minutes or until cells completely round up. Use a microscope to monitor the change in cell morphology.
6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, Cat. #0500).
7. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37ºC for another 1 to 2 minutes (no solution in the flask at this moment).
8. At the end of incubation, gently tap the side of the vessel to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.
9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.
10. Examine the vessel under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.

    Note: Use ScienCell T/E solution that is optimized to minimize cell damages due to over trypsinization.
11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Resuspend cells in culture medium.
12. Count and plate cells in a new gelatin-coated culture vessel with the recommended cell density.

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.