Mouse Embryonic Fibroblasts- Mitomycin C treated (MEF-mt)

Catalog Number: M7550

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]. These cells have been mitomycin treated to prevent further cell division prior to cryopreservation.

MEF cells from ScienCell Research Laboratories are isolated from day 13 mouse embryos. These cells are cryopreserved at primary culture and delivered frozen. Each vial contains 5 x 10^6 cells in 1 ml volume. MEF-mt are characterized by immunofluorescent imaging using antibodies to fibronectin. MEF-mt are negative for mycoplasma, bacteria, yeast and fungi.

Recommended Medium

It is recommended to use DMEM F12 (cat. no. 09411) 10% FBS (Cat. No. 09411) for the culturing of MEF-mt cells *in vitro*.

Product Use

MEF-mt 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°C waterbath and return them to culture as quickly as possible with minimal handling!

Set up culture after receiving the ordering:

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 37°C 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-mt.
4. Place the vial in a 37°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 a 1 ml eppendorf pipette gently re-suspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, gelatin coated culture vessels. A seeding density of 50,000 cells/cm^2 is recommended. Fill dishes to a total of 10mL with complete medium.
   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.
2. Change the medium every other day thereafter.

Caution: Handling animal derived products is potentially biohazardous. 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).