MEF Feeder Cells

CATALOG NUMBER: CBA-310

STORAGE: Liquid nitrogen
Note: For best results begin culture of cells immediately upon receipt. If this is not possible, store at -80ºC until first culture. Store subsequent cultured cells long term in liquid nitrogen.

QUANTITY & CONCENTRATION: 1 mL, 5 x 10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO

Background

Embryonic stem (ES) cells have been derived from the inner cell masses (ICM) of blastocysts in many species. They are capable of unlimited, undifferentiated proliferation on feeder cell layers and remain karyotypically normal and phenotypically stable. In addition, ES cells have the ability to differentiate into a wide variety of cell types *in vitro* and *in vivo*. In mES cell culture, the feeder layer can be replaced by the addition of LIF in the growth medium. However, LIF does not have the same effect on hES cell culture as mES. Therefore, both the derivation and maintenance of hES cells require the use of feeder cells.

Application

Embryonic Fibroblast (MEF) feeder cells are used for the maintenance of mouse or human ES cells in the undifferentiated state. The cells must be mitotically inactivated prior to the addition of ES cells, such as treatment with mitomycin C (2-4 hr, 10 µg/mL).

Quality Control

This cryovial contains at least 5.0 × 10^6 MEF feeder cells as determined by morphology, trypan-blue dye exclusion, and viable cell count. The MEF feeder cells are tested free of microbial contamination.

Medium

1. Culture Medium: D-MEM (high glucose), 10% fetal bovine serum (FBS), 0.1 mM MEM Non-Essential Amino Acids (NEAA), 2 mM L-glutamine, 1% Pen-Strep (optional)
2. Freeze Medium: 70% DMEM, 20% FBS, 10% DMSO

Methods

I. Establishing MEF Feeder Cell Cultures from Frozen Cells

1. Place 10 ml of complete DMEM growth medium in a 50-ml conical tube. Thaw the frozen cryovial of cells within 1–2 minutes by gentle agitation in a 37°C water bath. Decontaminate the cryovial by wiping the surface of the vial with 70% (v/v) ethanol.
2. Transfer the thawed cell suspension to the conical tube containing 10 mL of growth medium.
3. Collect the cells by centrifugation at 1000 rpm for 5 minutes at room temperature. Remove the growth medium by aspiration.

4. Resuspend the cells in the conical tube in 15 mL of fresh growth medium by gently pipetting up and down.
5. Transfer the 15 mL of cell suspension to a T-75 tissue culture flask. Place the cells in a 37°C incubator at 5% CO2.
6. Monitor cell density daily. Cells should be passaged when the culture reaches 95% confluence.

II. Freezing MEF Feeder Cells

1. Trypsinize cells and resuspend cell pellet in cold Freeze Medium at twice the desired final cell concentration.
2. Aliquot 1 mL of cells into sterile cryovials and place cryovials immediately into freezing container. Store overnight at -80 ^{0}C.
3. Transfer frozen vials to -135 ^{0}C freezer or liquid nitrogen.

III. Mitomycin C Treatment and Preparation of Feeder

1. Culture cells to 90% confluence. Wash it once with sterile PBS.
2. Add 10 µg/mL Mitomycin C (Sigma), incubate for 2 hrs.
3. Wash 3 times with sterile PBS to remove Mitomycin.
4. After dissociation by Trypsin, the Mitomycin-treated MEFs can be freezed and stored in liquid nitrogen, or used as feeder by plating them at 75 000 cells/cm^2 in gelatin-coated tissue culture dishes for one day.

Recent Production Citations

1. Sako, K. et al. (2025). Cells adapt to extracellular acidic pH through TM9SF3-mediated PI(4,5)P2 flop. *Nat Commun*. **16**(1):8852. doi: 10.1038/s41467-025-63524-w.
2. Natarajan, M. et al. (2021). In vitro propagation and cardiac differentiation of canine induced pluripotent stem cells on carbon nanotube substrates. *Tissue Cell*. doi: 10.1016/j.tice.2021.101571.
3. Schenke, M. et al. (2020). Analysis of Motor Neurons Differentiated from Human Induced Pluripotent Stem Cells for the Use in Cell-Based Botulinum Neurotoxin Activity Assays. *Toxins (Basel)*. **12**(5). pii: E276. doi: 10.3390/toxins12050276.

Warranty

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