iMEF (irradiated mouse embryonic fibroblasts)

ORDERING INFORMATION

Catalog Number: PSC001

Size: 5 vials; ~6 x 10^6 cells/vial

Cell Type: Irradiated primary mouse embryonic fibroblast cells at passage 3

Storage: Liquid nitrogen

Description

Mouse embryonic fibroblasts (MEF) were isolated from E13.5 CF1 embryos after removal of brain and visceral tissue. The MEF were then mitotically inactivated at passage 3 by gamma irradiation and cryopreserved.

Irradiated MEF are used to support the undifferentiated expansion of human (1) or mouse (2) embryonic stem (ES) cells in combination with the appropriate growth medium and growth factor supplements.

Cells Provided

Irradiated mouse embryonic fibroblasts - Approximately 3 x 10^7 cells total; 6 x 10^6 cells/vial.

Thawing of Cryopreserved Cells

Review the following protocol in detail before thawing the cells. Correct thawing procedures are critical and must be followed. For more details regarding the culture of pluripotent stem cells on iMEF, please visit the R&D Systems website at www.RnDSystems.com/go/iMEFprotocol.

1. Coat the appropriate sized plate(s) for the desired number of cells by covering the surface of the dish with 0.1% sterile gelatin for 15 minutes. For example, one vial of 6 x 10^6 iMEF can be plated on two 100 mm dishes, six 60 mm dishes, or two 6 well plates.
2. Warm the MEF Media (high glucose DMEM, 10% fetal bovine serum, 2 mM L-Glutamine) to 37 °C.
3. Thaw the desired number of vials of iMEF cells by quickly warming the cryotube(s) in a 37 °C water bath until the cells are just thawed. Immediately transfer the contents of one vial to a 15 mL conical tube containing at least 5 mL of pre-warmed MEF media. Rinse the vial with an additional 1 mL of media to ensure removal of all cells.
4. Spin at 200 x g for 5 minutes.
5. Remove the media and discard. Gently flick the pellet.
6. Immediately prior to plating cells, remove the 0.1% gelatin from the plates or wells.
7. Resuspend the iMEF cells in pre-warmed MEF Media, and transfer to the gelatin coated plates at a density of approximately 1 x 10^6 cells/60 mm plate.
8. Incubate overnight in a 37 °C, 5% CO_2 incubator.
9. The following day, the iMEF cells should appear as a monolayer covering the surface of the plate.

Storage

Store in liquid nitrogen for up to 2 years.

Quality Control

R&D Systems iMEF are tested for their ability to support undifferentiated growth of BG01V human ES cells as assessed by Oct4 and SSEA-4 expression. They are mycoplasma negative as tested by the MycoProbe™ mycoplasma detection kit (R&D Systems, Catalog # CUL001B) and negative for microbial contamination.

Precautions

This product contains 10% dimethylsulfoxide (DMSO).

References

1. Thomson, J.A. *et al*. (1998) Science **282**:1145.
2. Nagy, A. *et al*. (2003) *Manipulating the Mouse Embryo*. Cold Spring Harbor Laboratory Press.

R&D Systems, Inc.
1-800-343-7475