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Clonetics™ Irradiated Mouse Embryonic Fibroblast Cells

Technical Information & Instructions

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I. Introduction

Irradiated Mouse primary embryonic fibroblasts (iMEF) are dissociated from day 14 and 15 post-coitus CD-1 mouse embryos, expanded and then inactivated using gamma radiation. Each vial contains at least 2 million cells. iMEF are convenient and easy to use “feeder cells” commonly co-cultured with embryonic and pluripotent stem cell cultures.

II. Safety

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures.

WARNING: Handle as a potentially biohazardous material under Biosafety Level 1 containment. These cells are not known to contain an agent known to cause disease in healthy adult humans. These cells have not been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. If you require further information, please contact your site Safety Officer or Scientific Support.

III. Receiving Instructions

Unpack immediately! Packages may contain components with various storage requirements!

IV. Unpacking and storage instructions

1. Cells should be stored in liquid nitrogen. Do NOT store cells at -80°C. The cells are extremely temperature-sensitive and should be transferred to liquid nitrogen **immediately** upon arrival. Cells should be transported on dry ice or in a liquid nitrogen container. When transporting the cells on dry ice make sure that the vials are **completely** covered.

V. Preparation of culture medium

Media should be prepared fresh prior to use.

The recommended media for the iMEF cells is Lonza DMEM high glucose containing 10% FBS.

1. Thaw the FBS and pen/strep at room temperature.
2. Decontaminate the external surfaces of all supplement vials and the media bottle with ethanol or isopropanol.
3. Add the required amount to basal medium with a pipette:
   89 ml Dulbecco's modified eagle medium (DMEM)
   10 ml fetal bovine serum (heat inactivated)
   1 ml pen/strep
4. Filter the solution into a sterile container using a Nalgene 150 mL SFCA membrane 0.2 µm filter.

VI. Seeding Density and Performance

The recommended plating density for γ –Irradiated Mouse Embryonic Fibroblast is $5x10^4$ cells/mL or $2.5x10^4$ cells/cm^2.

VII. Thawing and Plating of iMEF Feeder Cells

All procedures are carried out under sterile conditions.

1. Coat desired culture surfaces with 0.1% sterile Gelatin (made from 2% stock {Sigma-G1393} in sterile Culture PBS. Place gelatin onto culture surfaces (125µL/cm^2) and incubate for 45 minutes at 37°C. Remove excess gelatin solution by aspiration and allow the coating to air dry in a laminar flow hood while vials of cells are being thawed and prepared for plating.

2. Remove a vial of cells from liquid nitrogen and place in a water bath pre-heated to 37°C.

   IMPORTANT: **Do not vortex the cells.** Keep the time between removing the vial from the liquid nitrogen tank and placing into a pre-heated water bath as short as possible.

3. After 2½ minutes, remove the vial from the water bath and disinfect the outside of the vial by wiping with 70% ethanol. Place the vial in a laminar flow hood. Proceed with the next step immediately after thawing.

4. Gently aspirate and dispense once using a P1000 pipettor, then transfer **entire contents** of vial to 15mL tube and add 8 mL pre warmed MEF Media dropwise onto the cells, while rotating the tube by hand. This should take approximately 2 minutes. **Important:** Do not add the entire volume of medium at once as this may result in osmotic shock. Centrifugation is not recommended.

5. Plate cells at recommended plating density. Suggested volumes for different plating formats are presented in Table 1 below.

6. Incubate the plated cells at 37°C in 5% CO_2 incubator.

Table 1

96-well plate	24-well plate	12-well plate	6-well plate
Per well add: 50 µL cell suspension + 150µL MFM	**Per well add:** 250 µL cell suspension + 750 µL MFM	**Per well add:** 500 µL cell suspension + 1.5 mL MFM	**Per well add:** 1.25 mL cell suspension + 1.75mL MFM
60 mm dish	**100 mm dish**	**25cm2 flask**	**75cm2 flask**
Per dish add: 2.5 mL cell suspension + 2.5 mL MFM	**Per dish add:** 6.9 mL cell suspension + 3.1 mL MFM	**Per dish add:** 3.2 mL cell suspension + 4.8 mL MFM	**Per dish add:** 9.4 mL cell suspension + 10.64 mL MFM

VIII. Maintenance

1. After 24 hours, replace media with fresh 37°C Mouse Fibroblast Media

2. Use the iMEF Feeder Cell wells within 3-4 days after plating

IX. Quality Control

The cells test negative for mycoplasma and bacteria. Each lot of iMEF is tested using mycoplasma PCR, bio-burden assay, and immunohistochemistry of vimentin expression. A certificate of analysis (COA) for each lot is shipped with each order. COAs for all other products are available upon request.

X. Product Warranty

Cultures have a finite lifespan *in vitro*. Lonza guarantees cell performance only when the approved media and supplements are used.

XI. Ordering Information

M-iFb-482	Irradiated Mouse embryonic fibroblasts	≥ 2 million cells in a 1 ml cell suspension
12-604F	DMEM, high glucose	500 ml
(Invitrogen) 26140-079 or equivalent	10% FBS, US origin, heat inactivated	100 ml
17-603E	Penicillin-streptomycin mixture	100 ml
(Sigma) G1393	Gelatin solution Type B, 2%	100 ml

When placing an order or for Scientific support, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics™ products, refer to the Lonza website or our current catalog. To obtain a catalog, additional information or technical service you may contact Lonza by web, e-mail, telephone, fax or mail.