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User Manual

OriCell™ Strain ICR Mouse Embryonic Fibroblasts (Irradiated)

Cat. No. MUIEF-01002

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CONTENT AND STORAGE

Product Name	Strain ICR Mouse Embryonic Fibroblasts (Irradiated)
Catalog No.	MUIEF-01002
Amount per Vial	1×10^6 Cells
Cryopreserved At	First Passage
Storage Condition	Liquid Nitrogen

CAUTION: **Please handle this product as a potentially biohazardous material. This product contains Dimethyl Sulfoxide (DMSO), a hazardous material, in the freezing medium.**

PRODUCT INTRODUCTION

Cyagen OriCell™ Strain ICR Mouse Embryonic Fibroblasts (Irradiated) are derived from qualified ICR mouse embryos (13.5 dpc), cultured as a monolayer, and cryopreserved at the first passage after *γ*-ray irradiation. These MEFs are used in culture mouse or human embryonic stem cells while maintaining their undifferentiated states.

A fibroblast is a type of cell that aremainly responsible for secreting the extracellular matrix. Mouse embryonic fibroblasts (MEFs) are usually applied as "feeder cells" in embryonic stem cells culture.

In addition, these cells have been tested for:

- Exogenous Factors: Including bacterium/fungus contamination, mycoplasma contamination, endotoxin contamination.
- Characteristics: Including post-thaw viability, verification of growth arrest, ability to Support ESC Growth.

This product is intended for laboratory research use only. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.

CELL CHARACTERISTICS AND IDENTITY

- Inactivated by gamma irradiation.

- Support the growth of mouse and human embryonic stem cells in their undifferentiated state.

GENERAL HANDLING PRINCIPLES

1. Aseptic handling of the product is necessary throughout.
2. Thawing the mouse embryonic fibroblasts (MEFs) should be performed at least one day before thawing the embryonic stem cells.
3. The MEFs should be used as soon as possible once thawed. We recommend the seeding density to be 2.5×10^4cells/cm^2.

GELATIN COATING OF TISSUE CULTURE VESSELS FOR MEFs

Materials Required:

- Gelatin Solution (Cat. No. GLT-11301)

Gelatin Coating of Tissue Culture Vessels

1. Add sufficient Gelatin Solution into the culture vessel to completely cover its base.
2. Swirl until Gelatin Solution coats the entire base of vessel. Let it sit for at least 30 minutes at room temperature.
3. Aspirate off all of the Gelatin Solution and allow the residual amount to evaporate by leaving the vessel sitting open in the laminar flow hood/biological safety cabinet for no more than 30 minutes.
4. Enclose the culture vessel once it has dried.

Note: **Gelatinized dishes or flasks can be stored at 4°C for no more than 2 weeks, provided they remain sterile.**

THAWING AND ESTABLISHING OriCell™ STRAIN ICR MEFs

Materials Required

- Gelatin Solution (Cat. No. GLT-11301)
- OriCell™ Strain ICR Mouse Embryonic Fibroblasts (Cat. No. MUIEF-01002)
- OriCell™ Mouse Embryonic Fibroblast Growth Medium (Cat. No. MUXEF-90011)

Thawing and Establishing MEFs

1. Pre-warm the OriCell™ MEF Growth Medium to 37°C.
2. Add 9 mL of OriCell™ MEFGrowth Medium to a 15 mL conical tube.
3. Remove the cryovial of OriCell™ Strain ICR MEFs from liquid nitrogen. Quickly thaw the vial in a 37°C water bath until the last ice crystal disappears. For optimal results, be sure to finish the thawing procedure within 3 minutes. Be careful not to submerge the entire vial. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

Note: **Results will be less than optimal if the cells are thawed for more than 3 minutes**

4. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% v/v ethanol.
5. Use a pipette to transfer the cells to the 15 mL conical tube containing OriCell™ MEF Growth Medium inside a biosafety cabinet. Be careful not to introduce any bubbles during the transfer process.
6. Rinse the vial with 1 mL of medium to reduce cell loss. Subsequently transfer this 1mL of cell suspension to the conical tube.
7. Gently mix the cell suspension by slowly pipetting up and down. Be careful not to introduce any bubbles.
8. Centrifuge the cell suspension at 250 x *g* for 5 minutes.
9. Carefully aspirate off as much of the supernatant as possible and add 3 mL of fresh OriCell™ MEF Growth Medium (pre-warmed to 37°C).
10. Gently resuspend the cells in OriCell™ MEF Growth Medium.
11. Seed the cells into 6-well plates pre-coated with Gelatin Solution (or other appropriate flasks) and add sufficient OriCell™ MEF Growth Medium. Gently rock the culture plate to evenly distribute the cells.

Note: **We recommend the seeding density to be 2.0-3.0×10^4cells/cm^2.**

12. Incubate at 37°C in a 5% CO_2 humidified incubator.
13. The next day, change the medium with fresh OriCell™ MEF Growth Medium (pre-warmed to 37°C).

Note:

1. **If the next day thawing of the embryonic stem cells is performed, the medium can be changed directly to embryonic stem cell growth medium.**
2. **Thawing the feeder cells should be performed at least one day before thawing embryonic stem cells.**
3. **The feeder cells should be used as soon as possible once thawed.**

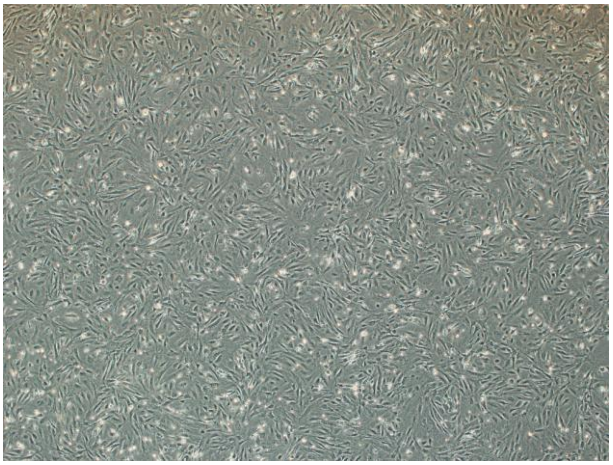

Fig. 1 Cyagen OriCell™ Strain ICR Mouse Embryonic Fibroblasts (Irradiated) plated on culture vessels coated with 0.1% gelatin.

APPENDIX

Troubleshooting

The table below lists some potential problems and solutions that may help you troubleshoot your MEF feeder cultures.

Problem	Cause	Solution
Low cell recovery rate	The storage condition does not meet the requirements	Purchase a replacement, and store in liquid nitrogen for long-term preservation.
	Thawing the cells takes too long time	Control the thawing procedure for no more than 3 minutes.
	Cells are incompletely recovered after thawing	After aspirating off medium, wash the tube with culture medium twice and transfer all cells to the dish.
	Cells are handled roughly	Care should be taken to avoid introducing bubbles during pipetting. Also avoid vortexing and high-speed centrifugation
	Medium is not pre-warmed	Warm medium to 37°C before recovery.
Slow cell growth	Mycoplasma contamination	Discard the cells in question, and disinfect the experimental environment before recovering.
	Over digestion	Wash the cells with PBS 2-3 times to remove serum prior to trypsinization (serum will inhibit the function of trypsin).
		Control the digestion time.
	Plating density is too low	Increase the plating density.

Related Products

Product	Catalog Number
Gelatin Solution	**GLT-11301**
OriCell™ Strain ICR Mouse Embryonic Fibroblasts	MUIEF-01002
OriCell™ Mouse Embryonic Fibroblast Growth Medium	**MUXEF-90011**