cyagen | OriCell

User Manual

OriCell™ ICR Mouse Embryonic Fibroblasts (Inactivated)

Catalog No. MUIEF-01002

We help you discover life

Introduction

Fibroblasts are cells that primarily secrete extracellular matrix and originate from mesenchymal cells in the embryonic mesoderm. Embryonic fibroblasts are commonly used as feeder cells for embryonic stem cell culture.

OriCell™ ICR Mouse Embryonic Fibroblasts (Inactivated) are derived from the trunk and limbs of 13.5-day ICR mouse embryos. These cells are cultured as monolayers, expanded to passage 1 (P1), then inactivated by γ-irradiation and cryopreserved. Mouse embryonic fibroblasts (MEFs) serve as feeder cells to support the culture of both mouse and human embryonic stem cells, helping to maintain their undifferentiated state.

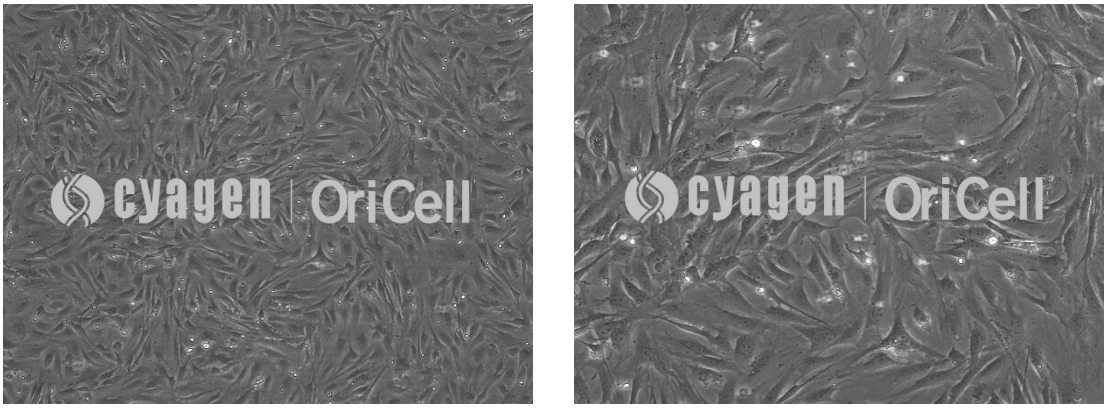
Note: **This product is intended for research use only and is not for diagnostic, therapeutic, clinical, household, or any other applications.**

When citing our products in academic publications, please use the following format: "OriCell™ [Product Name] + [Catalog Number], from Cyagen Biosciences."

Product Information

Product Name	OriCell™ ICR Mouse Embryonic Fibroblasts (Inactivated)
Catalog Number	MUIEF-01002
Amount of Cells	1×10^6 cells/vial; 3×10^6 cells/vial; 5×10^6 cells/vial
Passage Number	P1
Storage at	Liquid Nitrogen (-196 °C)

The Morphology of OriCell™ ICR Mouse Embryonic Fibroblasts (Inactivated)



QC

- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- Verified by cell recovery testing, with a post-thaw viability of >80%.
- Pass the test of cell ES supporting ability.

Please refer to "COA" for details.

General Handling Principles

1. Maintain strict aseptic technique. Ensure complete sterility throughout all procedures, particularly within the laminar flow hood and incubator.
2. Follow standardized protocols. Adhere strictly to the product manual. Implement rigorous control over experimental variables and include appropriate parallel controls.
3. Use high-quality consumables and reagents. This product requires culture vessels suitable for adherent growth, and the reuse of these vessels is not recommended. The reagents used must be validated for reliability, cell compatibility, and batch-to-batch consistency.
4. MEF cells should be thawed one day prior to the thawing of embryonic stem cells.

Note: **The cryopreservation medium of this product contains DMSO, which has potential risks. Please handle it carefully.**

Product Features

- After γ-ray inactivation, it loses the ability to proliferate.
- It supports the maintenance of the undifferentiated state and proliferation capacity of both mouse and human embryonic stem cells.

Gelatin Coating of Culture Vessels

Materials Required

- OriCell™ 0.1% Gelatin Solution (Cat. No.: GLT-11301)

Steps

Note: **For optimal adhesionen of γ-irradiated mouse embryonic fibroblasts (MEFs), culture vessels should be pre-coated with gelatin.**

1. Add an appropriate volume of 0.1% gelatin solution to the culture vessels (e.g., flask or dish), ensuring that the entire bottom surface is fully covered.
2. Gently rock the vessel to evenly distribute the gelatin across the surface.

   Note: **Gelatin coating must be applied evenly, and no air bubbles should remain on the bottom surface of the vessel.**

3. Incubate the gelatin-coated culture vessels in a biosafety cabinet (or laminar flow hood) for at least 30 minutes.
4. After 30 minutes, remove the gelatin solution and allow the vessels to air-dry completely before cell seeding.

   Note: **Gelatin-coated culture vessels may be stored at 4°C for up to two weeks under sterile conditions, provided that the gelatin does not dry completely.**

Thawing and Culturing of Cells

Materials Required

- OriCell™ ICR Mouse Embryonic Fibroblasts (Inactivated) (Cat. No.: MUIEF-01002)
- OriCell™ Complete Medium For Mouse Embryonic Fibroblasts (Cat. No.: MUXEF-90011)

Steps

Note: **If thawing is planned within 24 hours of receipt, store the cells in an ultra-low temperature freezer at -80 °C. For long-term storage (>24 hours) , keep them in liquid nitrogen. Before thawing, transfer the cells from liquid nitrogen to -80 °C and hold them there for 10 minutes. This will allow any residual liquid nitrogen to evaporate and prevent vial explosion.**

1. Pre-warm the water bath to 37 °C.
2. Warm the complete medium to 37 °C.
3. Add at least 5 mL of pre-warmed complete medium to a 15 mL centrifuge tube for subsequent use.
4. Remove the cryovial containing cells from the −80°C freezer, immerse it in the 37 °C water bath, and gently and quickly swirl to thaw the cryopreservation medium.

Note:

(1) Gently swirl the cryovial during thawing to ensure rapid and uniform thawing.

(2) Avoid submerging the cap in water to prevent contamination.

(3) Stop thawing in the water bath when only a single ice crystal (approximately 2 mm in diameter) remains, then continue gently swirling the vial until it is completely thawed.

5. Wipe the outer surface of the cryovial with 75% ethanol.
6. In a biosafety cabinet, open the cryovial and transfer the cell suspension to the prepared centrifuge tube using a Pasteur pipette.
7. Rinse the cryovial once with 1 mL of complete medium to collect residual cells and minimize loss.
8. Centrifuge the cell suspension at 250 × g for 4 minutes.

   Note: **Please calculate the corresponding rotational speed using the formula: RCF = 1.118 × 10^{-5} × r × RPM^2 (where RCF is the relative centrifugal force, r is the rotor radius in cm, and RPM is the rotational speed).**

9. Carefully remove the supernatant after centrifugation. Add 2 mL of complete medium, gently resuspend the cell pellet by pipetting up and down to mix thoroughly.
10. Seed the cells into a culture vessel at a density of 2.5 × 10^4 viable cells/cm^2 and add an appropriate volume of complete medium (Cat. No.: MUXEF-90011).
11. Gently swirl the flask to evenly distribute the cells, then incubate in a CO_2 incubator at 37 °C with 5% CO_2 and saturated humidity.

    Note: **Do not move or observe the cells within the first 2 hours after seeding, as this may impair cell adhesion, causing poor morphology, clumping, and uneven attachment.**

12. On the day after recovery, observe cell status and either replace the medium with fresh complete medium or passage the cells as needed.

Note:

1) If an excessive number of floating cells or any abnormal conditions are observed, investigate promptly and contact us for assistance.
2) MEF cells should be thawed one day prior to the thawing of embryonic stem cells.
3) If embryonic stem cells are to be thawed on the day of a scheduled MEF medium change, you may proceed directly with embryonic stem cell complete medium.
4) Thawed MEFs should be used within 3 days for optimal feeder layer performance.