cyagen | OriCell™

User Manual

OriCell™ ICR Mouse Embryonic Fibroblasts

Catalog No. MUIEF-01001

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 are derived from the trunk and limbs of 13.5-day ICR fetal mice.

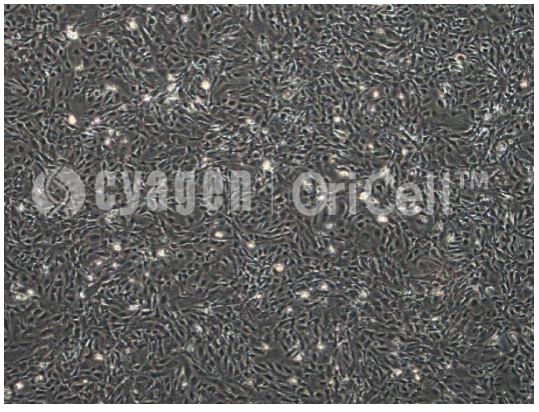
Note: **This product is only provided for further scientific research. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.**

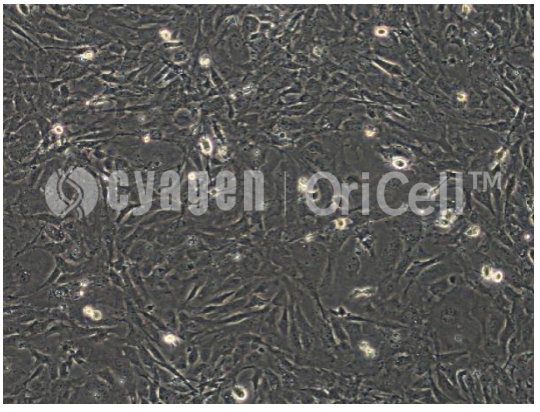
When citing our products in academic journals, please indicate "OriCell™ + Catalog Number, from Cyagen Biosciences (Guangzhou) Inc."

Product Information

Name	OriCell™ ICR Mouse Embryonic Fibroblasts
Catalog Number	MUIEF-01001
Amount of Cells	5×10^6 cells/vial
Passage Number	P1
Storage at	Liquid Nitrogen (-196°C)

The Shape of OriCell™ ICR Mouse Embryonic Fibroblasts





QC

- Pass the detection of bacteria, fungi, mycoplasma and endotoxins.
- Pass the viability examination. The viable rate is higher than 80%.
- The cell doubling time is less than 72 hours.

Please refer to "COA" for details.

General Handing Principles

1. Ensure that all equipment is kept clean and tidy.
2. Please follow the instructions.
3. Use suitable and reliable consumables and reagents.
4. OriCell™ ICR Mouse Embryonic Fibroblasts have limited ability to proliferate in vitro and cannot maintain their differentiation potential for a long time. OriCell™ ICR Mouse Embryonic Fibroblasts can be passaged for more than 5 times and still maintain all indicators qualified. But we always recommend using lower generation cells for scientific research.
5. Usually the inoculation density of OriCell™ ICR Mouse Embryonic Fibroblasts is (2.5~4) $\times 10^4$ live cells/cm^2.

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

Thawing and Establishing of Cells

Materials Required

- OriCell™ ICR Mouse Embryonic Fibroblasts (Cat. No.: MUIEF-01001)
- OriCell™ Complete Medium For Mouse Embryonic Fibroblasts (Cat. No.: MUXEF-90011)

Steps

Note: **If the received cells are thawed within 24 hours, they can be stored in a refrigerator at -80°C. If more than 24 hours, please store them in liquid nitrogen. Please take them out 10 minutes early before thawing and place them at -80°C to allow the liquid nitrogen in the tube to evaporate.**

1. Preheat the water bath at 37°C.
2. Warm the complete medium to 37°C.
3. Add more than 5mL of complete medium to a 15mL centrifuge tube for use.
4. Take the cells out of the -80°C refrigerator, put them in a 37°C water bath and shake them quickly to thaw the cryopreservation solution

   Note: **During the thawing process, the cryotube must be shaken to ensure that the solution thaws quickly and evenly.**

5. When shaking, please avoid water immersing the pipe cover to cause pollution.
6. When the cryopreservation solution has thawed into ice crystal with a diameter of about 2 mm, stop the water bath. Continue to shake the cryotube until the ice crystal melts thoroughly.
7. Wipe the outer surface of the cryotube with 75% ethanol.
8. Open the cryopreservation tube in the ultraclean bench, use a Pasteur pipette to suck the cell suspension, and transfer it to the prepared centrifuge tube.
9. Wash the cryotube once with 1mL of complete medium to collect residual cells to reduce loss.
10. Centrifuge the cell suspension at 250×g for 4 minutes.
11. Remove the supernatant after centrifugation. Add 2mL of complete medium, gently pipette the cell pellet, blow and mix thoroughly.
12. Inoculate the cells into a T25 flask or a culture container with an equivalent bottom area. Add enough complete medium, the total amount of medium in a T25 flask should not less than 5 mL.
13. Shake the cells well and incubate them in a CO_2 incubator at saturated humidity, 37°C, 5%

CO_2 inside.

Note: **Do not move or observe the cells within 2 hours of inoculation. This will seriously affect cell adhesion, resulting in poor shape, cell clumping, and uneven adhesion.**

14. On the next day of recovery, observe the cell status, and replace medium with fresh complete medium or passage.

    Note: **If you find lots of floating cells or other abnormal conditions, please investigate the cause in time and contact us.**

15. Then refresh the complete medium every 2 days until the cells have grown to 90% confluence, which requires passage generation.

Passaging of Cells

Materials Required

- OriCell™ 0.25% Trypsin-0.04% EDTA Solution (Cat. No.: TEDTA-10001)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)
- OriCell™ Complete Medium For Mouse Embryonic Fibroblasts (Cat. No.: MUXEF-90011)

Steps

1. Prewarm the complete medium, PBS and trypsin to 37°C.
2. Remove the medium in the culture container.
3. Wash the cells twice with PBS (approximately 3mL for T25 flask and 6mL for T75 flask). Please perform relatively slightly and wash thoroughly. Remove the PBS.
4. Add trypsin (approximately 1.5mL for T25 flask and 3mL for T75 flask), spread quickly to ensure full contact with the cells.
5. Observe the cells under a microscope. After about 70%~80% of the cells have shrunk and round, tap the outer wall of the culture vessel to remove the cells from the culture surface.

6. Add complete medium (approximately 3mL for T25 flask and 6mL for T75 flask) immediately, and then slightly shake the culture container to mix the medium and trypsin quickly to stop the digestion.
7. Use a pipette to suck up the cell suspension, pipetting the bottom surface of the culture container several times, and pipetting down as much as possible of the cells.

   Note: **The pipetting action should not be violent.**

8. Transfer the cell suspension to a centrifuge tube. Wash the container once with PBS (approximately 3mL for T25 flask and 6mL for T75 flask) to collect residual cells.
9. All the collected cell suspensions are centrifuged at 250×g for 4 minutes.
10. Remove the supernatant after centrifugation. Add 2mL of complete medium, gently pipette the cell pellet, blow and mix thoroughly.
11. Inoculate the cells into a suitable culture container at (2.5~4)×10^4 live cells/cm^2, or adjust the passage ratio according to the actual growth of the cells.

    Note: **OriCell™ ICR Mouse Embryonic Fibroblasts usually have a passage ratio of 1:3, and they will grow to reach confluence within 72 hours.**

12. Shake the cells well and incubate them in a CO_2 incubator at saturated humidity, 37°C, 5% CO_2 inside.
13. Then refresh the complete medium every 2 days until the cells have grown to 90% confluence, which requires passage generation or frozen.

    Note: **Under normal conditions, the growth time of OriCell™ ICR Mouse Embryonic Fibroblasts does not exceed 72 hours per generation, and there is no need to change the medium. Frequent fluid changes will destroy the built-up cellular micro-environment.**

Cryopreservation of Cells

Materials Required

- OriCell™ NCR Protein-free Cryopreservation Medium For General Use (Cat. No.: NCPF-10001)
- OriCell™ Cryopreservation Medium For General Use (Cat. No.: CYRO-10001)

Steps

1. If you choose OriCell™ Cryopreservation Medium For General Use, please put the freezing containers in the refrigerator at 4°C before next process.
2. The cells are cryopreserved after growing to appropriate density that can be passaged.
3. For cell digestion, please refer to OriCell™ ICR Mouse Embryonic Fibroblasts "Passaging Steps 1~9".
4. The cells are uniformly suspended with an appropriate amount of cryopreserved solution, then the supernatant is removed after centrifugation.
5. The cells are divided into cryopreservation tubes based on proportion or quantity.
6. If you choose OriCell™ Cryopreservation Medium For General Use, put the cryotube in the freezing containers, and then put the freezing containers in the -80°C refrigerator. If you choose OriCell™ NCR Protein-free Cryopreservation Medium For General Use, please disperse the cryopreservation tube directly into the refrigerator at -80°C.

   Note: **During the cryopreservation of cells, especially within 4 hours of the beginning, the refrigerator door should not be opened, which will seriously affect the survival rate of cells.**

7. After 8 hours, cells can be transferred to liquid nitrogen for long-term storage.

   Note: **We suggest that the storage time in the refrigerator at -80°C should not exceed 48 hours.**