cyagen | OriCell™

User Manual

OriCell™ Drugs Resistant Mouse Embryonic Fibroblasts (DR3, Inactivated)

Catalog No. MUDEF-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™ Drugs Resistant Mouse Embryonic Fibroblasts (DR3, Inactivated) are derived from the trunk and limbs of 13.5-day DR3 drug-resistant fetal mice. During monolayer culture, three selection antibiotics (G418, puromycin, and hygromycin) are added. After expansion to passage 1 (P1), the cells are inactivated by γ-irradiation and then 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.

.

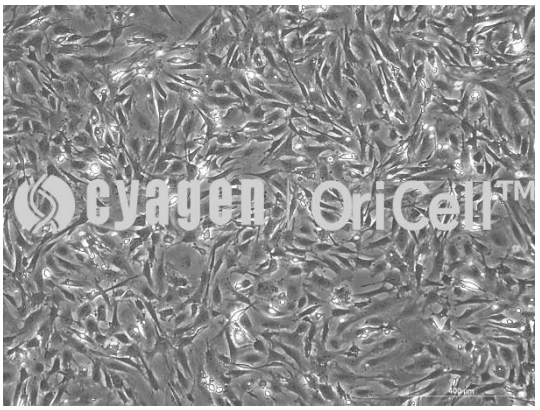
Note: **This product is only provided for further scientific research. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.**

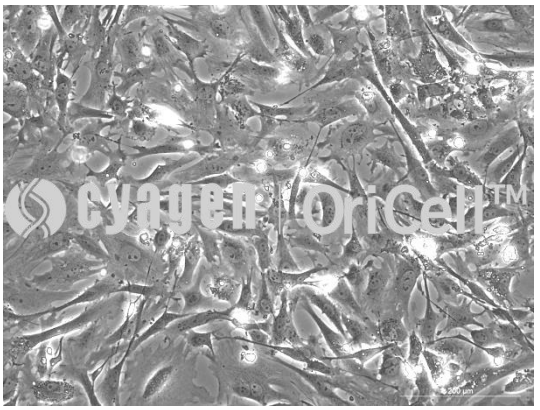
When citing our products in academic journals, please indicate "OriCell™ + Catalog Number, from Cyagen Biosciences (Guangzhou) Inc."

Product Information

Name	OriCell™ Drugs Resistant Mouse Embryonic Fibroblasts (DR3, Inactivated)
Catalog Number	MUDEF-01002
Number of Cells	1×10^6 cells/vial
Passage Number	P1
Storage at	Liquid Nitrogen (-196°C)

The Shape of OriCell™ Drugs Resistant Mouse Embryonic Fibroblasts (DR3, Inactivated)





QC

- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- Pass the viability examination. The viable rate is higher than 80%.
- Pass the test of cell ES supporting ability.

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. Resuscitate MEF the day before inoculation of embryonic stem cells.

Note: **The cryopreservation solution 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.

Culture Vessel Coated with 0.1% Gelatin

Materials Required

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

Steps

Note: **In order to make the first generation mouse embryonic fibroblasts irradiated by γ-rays more effectively adhere to the culture vessel, the surface of the culture vessel should be coated with gelatin.**

1. Add an appropriate amount of 0.1% gelatin to the culture flask to cover the entire bottom surface of the culture flask.
2. Shake the liquid to cover the entire bottom surface of the culture flask.
3. Place the culture flask covered with gelatin on the ultra-clean bench for at least 30 minutes.
4. After 30 minutes, remove the gelatin and wait for the flask to dry before it can be used to inoculate cells.

   Note: **Culture flasks coated with gelatin can be stored at 4°C for two weeks under sterile conditions also the gelatin is not evaporated to dryness.**

Thawing and Culturing of Cells

Materials Required

- OriCell™ Drugs Resistant Mouse Embryonic Fibroblasts (DR3, Inactivated) (Cat. No.: MUDEF-01002)
- 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 5 mL of complete medium to a 15 mL 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 1 mL 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 2 mL 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.