cyagen | OriCell™

User Manual

OriCell™ 129 Mouse Embryonic Stem Cells

Catalog No. MUAES-01001

We help you discover life

Introduction

Embryonic stem cells (ESC) are pluripotent stem cells derived from the inner cell mass of a blastocyst. It has the ability to differentiate into ectoderm, endoderm and mesoderm, and can differentiate into various types of cells. Unlike other stem cells, embryonic stem cells have the ability to proliferate indefinitely. The plasticity and unlimited proliferation of embryonic stem cells make it a hotspot in regenerative medicine and tissue engineering research.

OriCell™ 129 Mouse Embryonic Stem Cells remain normal diploid after being expanded and cultured in vitro, expressing special markers of embryonic stem cells, and can form embryoid bodies (EB) when cultured in vitro, and teratomas can be formed in vivo experiments. 129 mouse embryonic stem cells are a powerful tool for basic and applied research in many fields, including development and regulation research, regenerative biology, and potential treatment methods. In addition, genetically modifying embryonic stem cells and introducing them into the mouse germline is a very effective method to obtain genetically modified mice.

OriCell™ 129 Mouse Embryonic Stem Cells are derived from the inner cell mass of the 129 mouse 3.5-day blastocyst stage, using OriCell™ mouse embryonic stem cell culture medium and cultured on γ-ray-treated MEF (mouse embryonic fibroblasts) .

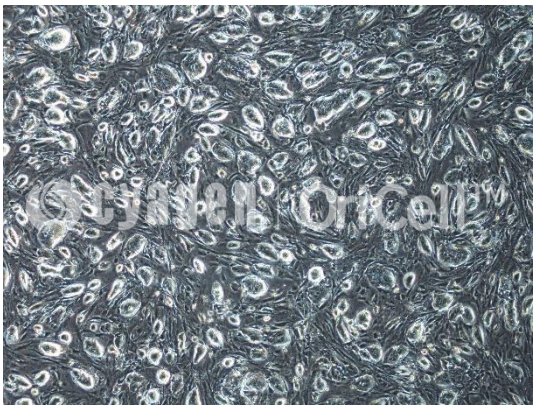
Note: **This product is only provided for further scientific research. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.**

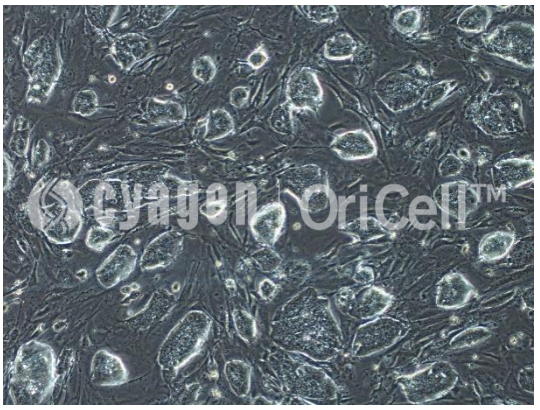
When citing our products in academic journals, please indicate "OriCell™ + Catalog Number, from Cyagen Biosciences (Guangzhou) Inc."

Product Information

Name	OriCell™ 129 Mouse Embryonic Stem Cells
Catalog Number	MUAES-01001
Amount of Cells	1×10^6 cells/vial
Passage Number	P20
Storage at	Liquid Nitrogen (-196°C)

The Shape of OriCell™ 129 Mouse Embryonic Stem Cells





QC

- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- Pass the viability examination. The viable rates is higher than 50%.
- The cell doubling time is less than 72 hours.
- By immunofluorescence detection, it expresses Oct4, SSEA-1 and Nanog (≥ 90%), but does not express SSEA-3 and SSEA-4 (≤ 5%).

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. If using feeder cells for culture, it is recommended to use OriCell™ ICR Mouse Embryonic Fibroblasts (Inactivated) (Cat. No.: MUIEF-01002).
5. If using serum-free and feeder-free culture conditions, it is recommended to use OriCell™ Serum Free Medium For Mouse Embryonic Stem Cells (Type II, Feeder-free) (Cat. No.: MUXES-90061).
6. Usually the inoculation density of OriCell™ 129 mouse embryonic stem cells is (1~2) $\times 10^4$ live cells/cm^2.

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

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 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 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 1 mL of complete medium, gently pipette the cell pellet, blow and mix thoroughly.
12. Inoculate the cells into a culture vessel at a density of 2.5×10^4 viable cells/cm^2, add a sufficient amount of embryonic fibroblast complete medium, and gently shake the cell culture vessel to distribute the cells evenly.
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.**
- **MEF cells need to be resuscitated the day before embryonic stem cell resuscitation.**
- **If the mouse embryonic stem cells will be resuscitated on the day of MEF cell replacement, they can be directly replaced with mouse embryonic stem cell culture medium.**
- **MEF after resuscitation should be used within 3 days.**

Thawing and Culturing of Cells

Materials Required

- OriCell™ 129 Mouse Embryonic Stem Cells (Cat. No.: MUAES-01001)
- OriCell™ Complete Medium For Mouse Embryonic Stem Cells (Cat. No.: MUXES-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 culture vessel at a density of 2.5×10^4 viable cells/cm^2, add a sufficient amount of embryonic fibroblast complete medium, and gently shake the cell culture vessel to distribute the cells evenly.
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.**

15. 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.**

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 Stem Cells (Cat. No.: MUXES-90011)
- OriCell™ Complete Medium For Mouse Embryonic Fibroblasts (Cat. No.: MUXEF-90011)
- Culture Vessel Coated with MEF

Steps

Note:

1) Normally, the cells can be cultured for 48 hours before the next passage.

2) Mouse embryonic stem cells must be passaged when the following conditions occur:

- The clones of mouse embryonic stem cells are relatively large, and they have differentiated or are about to differentiate;
- Although mouse embryonic stem cells did not show obvious differentiation, due to the problem of passaging and inoculation density, inter-clonal fusion occurred or was about to occur.

Experimental Preparation: Prepare feeder cells 1 to 3 days in advance (see Recovery of ICR Mouse Embryonic Fibroblasts).

1. Prewarm complete medium, PBS and trypsin to 37°C.
2. Remove the medium in the culture container.
3. Wash the cells twice with PBS (approximately 3 mL for T25 flask and 6 mL for T75 flask). Please perform relatively slightly and wash thoroughly. Remove the PBS.
4. Add trypsin (approximately 1.5 mL for T25 flask and 3 mL 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 For Mouse Embryonic Fibroblasts (approximately 3 mL for T25 flask and 6 mL 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 3 mL for T25 flask and 6 mL for T75 flask) to collect residual cells.

9. All the collected cell suspensions are centrifuged at 250×g for 4 minutes.

Note: **During centrifugation, replace the MEF prepared in advance with Complete Medium For Mouse Embryonic Stem Cells (preheated to 37°C).**

10. Remove the supernatant after centrifugation. Add 2 mL of Complete Medium For Mouse Embryonic Stem Cells, gently pipette the cell pellet, blow and mix thoroughly.
11. Inoculate the Mouse embryonic stem cells into a suitable culture container at $(1.0\sim2.0)\times10^4$ live cells/cm^2, or adjust the passage ratio according to the actual growth of the cells.
12. Shake the cells well and incubate them in a CO_2 incubator at saturated humidity, 37°C, 5% CO_2 inside.
13. On the next day of passage, observe the cell status. If more floating cells are found, the medium should be changed.
14. After the cell mass grows to a suitable size, it needs to be passaged or frozen.

Differentiation of Cells

Materials Required

- OriCell™ Embryoid Body (EB) Formation Medium (Cat. No.: MUXES-90051)

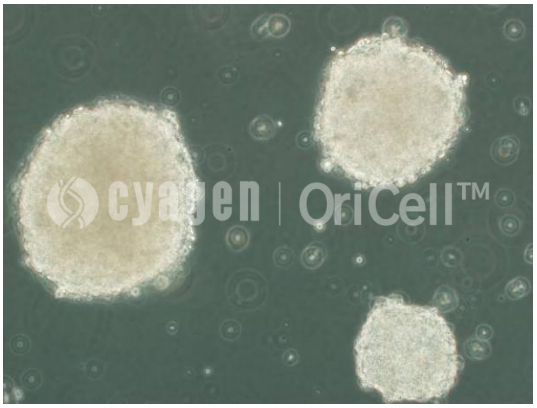
OriCell™ 129 mouse embryonic stem cells can form embryoid bodies under in vitro culture conditions, and teratomas can be formed in vivo experiments. The formation of embryoid bodies (EB) is the main step of embryonic stem cell differentiation. In the absence of a mouse embryonic fibroblast (MEF) feeder layer, embryonic stem cells will spontaneously differentiate to form three-dimensional aggregates when stimulated by the EB formation medium. This structure is conducive to cell interactions, such as cell-to-cell contact and the establishment of gap junctions.

Steps for Induction of Embryoid Bodies

1. Prepare a 100 mm cell culture dish coated with gelatin. See "Culture Vessel Coated with 0.1% Gelatin".
2. Prepare a T75 culture flask with embryonic stem cells (about 1×10^7 cells). When the cells grow to the logarithmic phase, embryoid bodies can be prepared.
3. Digest the cells. (Note: The cells need to be digested into individual cells to ensure their homogeneity. The digestion was terminated by adding embryoid body formation medium.)
4. Collect the cells, transfer the cell suspension to a 15 mL centrifuge tube, 250×g, and centrifuge for 5 min.
5. Carefully discard the supernatant.
6. Add embryoid body formation medium for resuspension, inoculate about 5×10^6 cells into a gelatin-coated culture vessel, and add about 8 mL of embryoid body formation medium.
7. Put it in an incubator at 37°C, 5% CO_2, and saturated humidity for 40 minutes to remove MEF.
8. Take out the culture dish and gently collect non-adherent cells (mostly embryonic stem cells) for the next step. If there is still lots of residual MEF in the collected suspension, repeat the above 5~8 steps once to remove MEF.
9. After counting, adjust the cell density to 5.5×10^4 viable cells/mL, inoculate them in 60 mm cell culture dishes, and add 5 mL of cell suspension to each dish. it is necessary to use a container that has not been Tissue culture treated (TC treatment) and is suitable for suspension cell culture.
10. Put it in an incubator at 37°C, 5% CO_2, and saturated humidity for 48 hours.
11. Two days later, a spherical suspended embryoid body of uneven size can be seen. At this time, most of the embryoid body is small, the embryonic body is transparent, and the refraction is good. The fluid can be changed by centrifugation (140×g, centrifugation for 1 min).
12. After changing the medium, inoculate it in a new culture dish (not treated with TC), and place it in a CO_2 incubator at 37°C, 5% CO_2, and saturated humidity for 72 hours.
13. In the next three days, the mesoblastoid body gradually enlarges, and the embryoid body should be transparent and compact under high magnification. There may be a tendency of aggregation and adhesion between individual embryoid bodies.

14. After 5 days of embryoid body suspension culture, 140×g, centrifugation for 1 min, resuspend the embryoid body with embryoid body formation medium, inoculate it in a 24-well plate, 1 mL/well, inoculate a total of 8 wells, the number of embryoid bodies in each hole is about 10-20.
15. Continuously cultivate in a 37°C, 5% CO_2, saturated humidity incubator for 14 days, change the medium every 2 to 3 days, observe the embryoid body differentiation, if the differentiation is ideal,the spontaneous pulsation of differentiated myocardium can be observed after 5-7 days of differentiation.
16. After 14 days, using immunofluorescence method to detect the differentiation of endoderm, mesoderm and ectoderm.

The Picture of Embryoid Bodies (EB) Derived from OriCell™ 129 Embryonic Stem Cells



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)

OriCell™ NCR Protein-free Cryopreservation Medium For General Use is a protein-free, ready-to-use cryopreservation solution. No protein, clear composition, suitable for cryopreservation of stem cells and

primary cells, without affecting cell growth and differentiation potential. No program is required, and the cells can be directly placed in a refrigerator at -80°C, making the operation more convenient and faster.

Steps

Note:

- **The cells need to be replaced with fresh complete medium 24 hours before cryopreservation.**
- **After the cells grow to a density that can be passaged, they are ready to be cryopreserved.**
- **For cell digestion, please refer to Steps 2~10 of OriCell™ 129 Mouse Embryonic Stem Cells Passaging.**

1. The cells are uniformly suspended with an appropriate amount of cryopreserved solution, then the supernatant is removed after centrifygation.
2. The cells are divided into cryopreservation tubes based on proportion or quantity.
3. If you choose OriCell™ Cryopreservation Medium For General Use, please put the freezing containers in the refrigerator at 4°C before next process.
4. 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.
5. If you choose OriCell™ NCR Protein-Free Cryopreservation Medium, 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.**

6. 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.**