cyagen | OriCell

User Manual

OriCell™ C57BL/6 Mouse Embryonic Stem Cells With GFP

Catalog No. MUBES-01101

We help you discover life

Introduction

Embryonic stem cells (ESCs) are pluripotent stem cells derived from the inner cell mass of the blastocyst. They possess the capacity to differentiate into ectoderm, endoderm, and mesoderm lineages, and can further develop into various cell types. Unlike other types of stem cells, embryonic stem cells have the ability to proliferate indefinitely, exhibiting unlimited self-renewal capacity. Their pluripotency and infinite proliferative potential make them a focal point in regenerative medicine and tissue engineering research.

After in vitro expansion, OriCell™ C57BL/6 Mouse Embryonic Stem Cells with GFP maintain a normal diploid karyotype and express characteristic embryonic stem cell markers. They can form embryoid bodies (EBs) in vitro and give rise to teratomas in vivo. These cells serve as a valuable tool for basic and applied research across various fields, including developmental and regulatory biology, regenerative biology, and potential therapeutic approaches. In addition, genetic modification of embryonic stem cells followed by their introduction into the mouse germline represents an efficient strategy for generating genetically modified mice.

OriCell™ C57BL/6 Mouse Embryonic Stem Cells are derived from the inner cell mass of C57BL/6 mouse blastocysts at embryonic day 3.5 (E3.5). They are maintained on γ-irradiated mouse embryonic fibroblast (MEF) feeder layers using OriCell™ Complete Medium for Mouse Embryonic Stem Cells (Cat. No.: MUXES-90011). After transfection, they stably express the GFP reporter gene, are expanded through serial passaging, and finally cryopreserved for long-term storage.

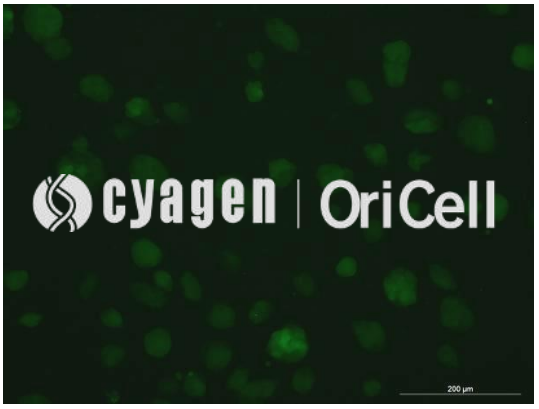
Note: **This product is intended for research use only and is not for diagnostic, therapeutic, clinical, household, or any other applications.**

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™ C57BL/6 Mouse Embryonic Stem Cells With GFP
Catalog Number	MUBES-01101
Number of Cells	1×10^6 cells/vial
Passage Number	P23
Storage at	Liquid Nitrogen (-196 °C)

The Morphology of OriCell™ C57BL/6 Mouse Embryonic Stem Cells with GFP



QC

- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- Verified by cell recovery testing, with a post-thaw viability of > 50%.
- Verified by cell cycle analysis, with a doubling time < 72 h.
- Verified by flow cytometry: Positive for Oct4, SSEA-1 and Nanog (≥ 70%); Negative for SSEA-3 and SSEA-4 (≤ 5%).

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. Optimize seeding density and subculture. The recommended seeding density for OriCell™ C57BL/6 Mouse Embryonic Stem Cells with GFP is (1–2) × 10^4 viable cells/cm^2. Since cell growth is highly dependent on donor characteristics and culture conditions, we recommend adjusting the split ratios based on the actual performance of each specific lot and passage.

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

Product Recommendation

Culture System	Product Recommendation	Cat. No.
Feeder-dependent	OriCell™ ICR Mouse Embryonic Fibroblasts (Inactivated)	MUIEF-01002
Serum-free, feeder-dependent	OriCell™ Serum Free Medium For Mouse Embryonic Stem Cells (Type I, With Feeder Layer)	MUXES-90062
Serum-free, feeder-free	OriCell™ Serum Free Medium For Mouse Embryonic Stem Cells (Type II, Feeder-free)	MUXES-90061

* For culture system transition, please refer to the corresponding instructions.

Gelatin Coating of Culture Vessels

Materials Required

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

Steps

Note: **To facilitate the attachment of γ-irradiated primary mouse embryonic fibroblasts (MEFs), it is strongly recommended to pre-coat the culture vessel with gelatin.**

1. Add an appropriate volume of 0.1% gelatin solution to the culture vessels (e.g., flasks or dishes).
2. Gently swirl the vessels to evenly distribute the gelatin over the entire bottom surface.
3. Place the gelatin-coated vessels inside a biosafety cabinet (laminar flow hood) for at least 30 minutes.
4. After 30 minutes, aspirate the gelatin solution and allow the vessels to air-dry completely before seeding.

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

Thawing and Culturing of MEF 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 swirl to facilitate rapid thawing.

   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 1 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 sufficient complete medium (Cat. No.: MUXEF-90011).
11. Gently swirl the culture vessel 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 (Cat. No.: MUXES-90011).**
    4) **Thawed MEFs should be used within 3 days for optimal feeder layer performance.**

Thawing and Culturing of Target Cells

Materials Required

- OriCell™ C57BL/6 Mouse Embryonic Stem Cells With GFP (Cat. No.: MUBES-01101)
- OriCell™ Complete Medium For Mouse Embryonic Stem Cells (Cat. No.: MUXES-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 swirl to facilitate rapid thawing.

   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 pre-coated with a MEF feeder layer and containing fresh medium at a density of (1-2) × 10^4 viable cells/cm^2.
11. Gently swirl the culture vessel 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.**

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

    Note: **If an excessive number of floating cells or any abnormal conditions are observed, investigate promptly and contact us for assistance.**

14. Replace the complete medium every 24 h until cell clusters reach appropriate size for passaging.

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 Vessels Coated with MEF

Steps

Note:

- **Typically, cells are ready for the next passage after 48 h of culture.**
- **Mouse embryonic stem cells must be passaged if any of the following occur:**
  - **(1) The colonies of mouse embryonic stem cells become large and show signs of differentiation or are about to differentiate.**
  - **(2) Although no obvious differentiation is observed, colonies are merging or about to merge (confluence) due to high seeding density during the previous passage.**

Experimental Preparation: **Prepare feeder cells 1–3 days in advance (see Thawing and Culturing of MEF Cells).**

1. Pre-warm the complete medium, PBS and trypsin to 37 °C.
2. Remove the medium in the culture vessel of mouse embryonic stem cells.
3. Gently wash the cells twice with PBS (approximately 3 mL for a T25 flask or 6 mL for a T75 flask). Ensure thorough washing but avoid excessive force. Then remove the PBS.
4. Add trypsin (approximately 1.5 mL for a T25 flask or 3 mL for a T75 flask), quickly spread it to ensure full coverage of the cell layer.

5. Observe the cells under a microscope. When approximately 70% to 80% of the cells have shrunk and become round, gently tap the outer wall of the culture vessel to detach the cells from the surface.
6. Immediately add complete medium (Cat. No.: MUXEF-90011) (approximately 3 mL for a T25 flask or 6 mL for a T75 flask), then gently swirl the culture vessel to mix the medium and trypsin, stopping the digestion process.
7. Collect the cell suspension using a pipette, gently pipetting along the bottom of the vessel several times to ensure maximal recovery of the cells.

   Note: **Pipetting should be performed gently to avoid creating excessive bubbles, as this may cause cell damage or loss.**

8. Transfer the cell suspension to a centrifuge tube. Rinse the culture vessel once with PBS (approximately 3 mL for a T25 flask or 6 mL for a T75 flask) to collect residual cells and add the wash to the centrifuge tube.
9. Centrifuge all collected cell suspensions at 250 × g for 4 minutes.

   Note: **During centrifugation, replace the medium in MEF feeder layer-coated plates with Complete Medium For Mouse Embryonic Stem Cells (Cat. No.: MUXES-90011, pre-warmed to 37 °C).**

10. Remove the supernatant after centrifugation. Add 2 mL of complete medium (Cat. No.: MUXES-90011), gently resuspend the cell pellet by pipetting up and down to thoroughly mix.
11. Seed the cells into a culture vessel pre-coated with a MEF feeder layer and containing fresh medium at a density of (1 - 2) × 10^4 viable cells/cm^2.

    Note: **We recommend manual cell counting where possible to obtain an accurate concentration for seeding. If precise counting is not feasible, subculturing at an appropriate split ratio is preferred. It is essential to seed cells at an appropriate density. Overly low density will result in slow cell growth, whereas overly high density may lead to spontaneous differentiation of embryonic stem cells. Please adjust the subculture ratio based on the actual growth of the cells.**

12. Gently agitate the vessel to ensure uniform cell distribution and place it in an incubator at 37 °C, 5% CO_2, and saturated humidity.
13. On the day after passaging, observe the cell condition. If a significant number of floating cells are present, replace the culture medium.
14. Once the colonies have grown to a suitable size, they are ready for passage or cryopreservation.

Cryopreservation of Cells

Materials Required

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

*Special Note: OriCell™ NCR Protein-Free Cryopreservation Medium For General Use (Cat. No.: NCPF-10001) is a ready-to-use, protein-free cryopreservation medium. It enables direct −80 °C freezing without the need for controlled-rate cooling, while maintaining cell growth and differentiation potential.

Steps

Note: **Perform a complete medium change 24 h before cryopreservation.**

1. Cells should be cryopreserved once they reach an appropriate density suitable for passaging.
2. For cell digestion, please refer to passaging steps 2 - 10 above.
3. After centrifugation, aspirate the supernatant and gently resuspend the cell pellet in an appropriate volume of cryopreservation medium.

4. Aliquot the cells into cryovials according to the desired cell number or proportion.

   Note: **If accurate cell counting is not feasible, we recommend aliquoting cells proportionally for freezing. Prolonged exposure to non-culture conditions will significantly compromise cell viability. Maintain the cells at 4 °C during counting to minimize metabolic activity and preserve cell integrity.**

5. When using any of the recommended NCR cryopreservation media above, cryovials can be placed directly into a -80 °C freezer.

   Note: **Avoid opening the freezer door during the first 4 hours of freezing, as temperature fluctuations can adversely affect cell viability.**

6. After approximately 8 hours, transfer the cryovials to liquid nitrogen for long-term storage.

   Note: **Do not store the cryovials at -80 °C for more than 48 hours.**