cyagen | OriCell

# User Manual

## OriCell™ Serum Free Medium

## For Mouse Embryonic Stem Cells

## (Type II, Feeder-free)

**Catalog No. MUXES-90061**

*We help you discover life*

## Introduction

OriCell ™ Serum Free Medium For Mouse Embryonic Stem Cells (Type II, Feeder-free) has been meticulously optimized by our R&D team. It consists of a serum-free basal medium, precisely formulated serum-free supplements, and other essential components.

This product is specifically designed for feeder-free, serum-free culture of mouse embryonic stem cells (mESCs). It can maintain the good proliferation characteristics and highly undifferentiated pluripotency of mouse embryonic stem cells in a serum-free and feeder-free growth environment, while preserving normal karyotype stability over extended culture.

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

| Components | Catalog Number | Volume | Storage |
|---|---|---|---|
| OriCell™ Basal Medium For Cell Culture | BKOD-03011 | 480 mL | 4 °C |
| OriCell™ Serum Replacements For Mouse Embryonic Stem Cells Serum Free Culture (Type II) | MUXES-11061 | 15 mL | -20 °C |
| OriCell™ Supplements For Mouse Embryonic Stem Cells Serum Free Culture (Type II) | MUXES-04061 | 5 mL | -20 °C |

## QC

- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- Pass the detection of osmotic pressure and pH.
- Pass the detection of product quality.

**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 instructions. Implement rigorous control over experimental variables and include appropriate parallel controls.

3. Ensure proper storage and use. Store all components according to specified conditions and use them promptly to ensure optimal performance.
4. Aliquot for long-term storage. If the entire volume will not be used up immediately, prepare the medium in batches according to the specified component volume ratios and store in aliquots.

## Product Stability and Storage Conditions

1. All components must be stored away from light.
2. The basal medium has a shelf life of 1 year and should be stored at 4 °C. Other components have a shelf life of 2 years and should be stored at -20 °C.
3. Once prepared, the medium has a shelf life of 1 month when stored at 4 °C. The shelf life may be extended up to a maximum of 45 days, provided that culture conditions remain stable, the container is properly sealed, and repeated temperature fluctuations are avoided.
4. Use all components before the expiration dates. Expired components may severely compromise culture performance.

## Preparation of Complete Medium

### Materials Required

- OriCell™ Serum Free Medium For Mouse Embryonic Stem Cells (Type II, Feeder-free) (Cat. No.: MUXES-90061)
- Clean, sterile, and stable quality disposable consumables (pipettes, pipette tips, centrifuge tubes, etc.)

- Clean sealing film
- Aluminum foil and other light-avoiding materials

## Steps

1. At least 1 hours before preparation, place the OriCell™ Serum Replacements For Mouse Embryonic Stem Cells Serum Free Culture (Type II) (Cat. No.: MUXES-11061) and OriCell™ Supplements For Mouse Embryonic Stem Cells Serum Free Culture (Type II) (Cat. No.: MUXES-04061) in a refrigerator at 4 °C to allow it to thaw completely.
2. Carefully wipe the outer packaging of all components with 75% ethanol. Open the package inside a clean bench (laminar flow hood).
3. Add all culture supplements (Cat. No.: MUXES-11061 and MUXES-04061) to OriCell™ Basal Medium (Cat. No.: BKOD-03011).
4. Tighten the cap of the basal medium bottle and swirl the bottle gently to mix thoroughly.

   Note:

   **1) If the medium will not be used up immediately, we recommend preparing in batches. Please prepare the required amount according to the ratio of each component in the kit. Any remaining components must be stored according to their respective storage conditions and should not be subjected to multiple freeze-thaw cycles.**

   **2) Please choose whether to add antibiotics (e.g., Penicillin/Streptomycin) according to your own needs. If required, it should be prepared or purchased separately.**

5. Seal the bottle with Parafilm, wrap it in aluminum foil to protect from light, and label it with the product name, preparation date, and other relevant information.

   Note: **All components in OriCell™ Serum Free Medium For Mouse Embryonic Stem Cells (Type II, Feeder-free) are strictly aseptically controlled. Under normal circumstances, we do not recommend sterilization again. If there is a risk of contamination during the preparation process, the complete**

medium can be filtered and sterilized.

## Gelatin Coating of Culture Surfaces

### Materials Required

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

### Steps

Note: **To promote optimal growth of mouse embryonic stem cells under serum-free conditions, pre-coating the culture vessel with gelatin is strongly recommended.**

1. Add sufficient 0.1% gelatin solution to completely cover the bottom of the culture vessel.
2. Incubate at room temperature for at least 30 minutes.
3. If not used immediately, seal the culture vessel with Parafilm and store at 4 °C. Use within one week.
4. Before use, aspirate the gelatin solution completely and allow the surface to air-dry.

## Transition of Culture Conditions

### 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™ Serum Free Medium For Mouse Embryonic Stem Cells (Type I, With Feeder Layer) (Cat. No.: MUXES-90062)
- OriCell™ Serum Free Medium For Mouse Embryonic Stem Cells (Type II, Feeder-free) (Cat. No.: MUXES-90061)

Note: To ensure optimal cell growth, it is strongly recommended to use OriCell™ Serum-Free Medium for Mouse Embryonic Stem Cells (Type I, with Feeder Layer) (Cat. No.: MUXES-90062) to transition the cells from a feeder-dependent, serum-containing culture system to a feeder-dependent, serum-free culture system. After 1–2 passages, the cells can be further adapted to a feeder-free, serum-free system.

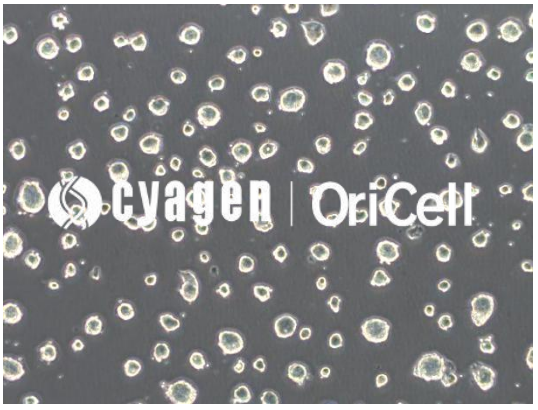
## Steps (Transition from Feeder-dependent to Feeder-free)

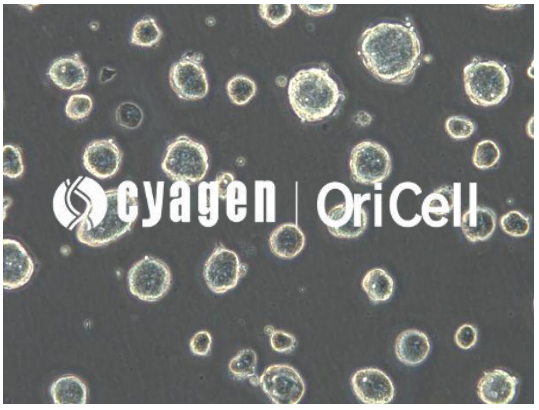
1. Digest mouse embryonic stem cells cultured in Serum Free Medium Type I (Cat. No.: MUXES-90062).
2. Centrifuge the cell suspension at 250 × g for 5 minutes and collect the cell pellet.
3. Resuspend the cells in the original medium, and transfer the entire cell suspension to a gelatin-coated culture vessel. Place it in a 37°C, 5% $CO_2$ incubator with saturated humidity. This step is intended to remove feeder cells from the original cell population as completely as possible.
4. After 30-40 minutes (timing may be adjusted based on the extent of feeder cell attachment), carefully collect the supernatant and centrifuge at 250 × g for 5 minutes.

   Note: During culture in Serum Free Medium Type I (Cat. No.: MUXES-90062), feeder cells may form a detachable cell layer. As a result, most feeder cells can be removed during the dissociation process. Therefore, Steps 3 and 4 may be omitted, and the procedure may proceed directly to Step 5.

5. Aspirate the supernatant and resuspend the cells in Serum-Free Medium Type II (Cat. No.: MUXES-90061). Seed the cells into gelatin-coated culture vessels at the desired density.
6. Add an appropriate volume of Serum Free Medium Type II (Cat. No.: MUXES-90061) and incubate at 37 °C with 5% $CO_2$ in a humidified incubator.
7. On the following day, observe the cells cultured in the Serum Free Medium Type II (Cat. No.: MUXES-90061), and replace with fresh Type II medium to remove residual feeder cells and any dead cells that failed to adapt to serum-free culture conditions.

C57BL/6 Mouse ESCs After 3 Days of Feeder-free, Serum-free Culture




## Passaging Protocol (Serum-free, Feeder-free)

### 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™ Serum Free Medium For Mouse Embryonic Stem Cells (Type II, Feeder-free) (Cat. No.: MUXES-90061)

### Steps

1. Warm the Serum Free Medium Type II (Cat. No.: MUXES-90061), PBS (Cat. No.: PBS-10001) and trypsin (Cat. No.: TEDTA-10001) to 37 °C before use.
2. Aspirate the medium from the mouse embryonic stem cell culture vessel.
3. Wash the cells 2–3 times with 1 × PBS to remove any residual medium.
4. Add trypsin (approximately 1 mL for a 35 mm culture dish or 2–3 mL for a 100 mm culture dish).

Gently swirl the vessel to ensure complete coverage of the cell layer and incubate until the cells begin to detach.

Note: **Digestion time may vary depending on the trypsin activity/titer used in different laboratories. Determine the optimal incubation time by monitoring the cells closely under a microscope.**

5. Add at least 2 mL of complete medium (containing serum, Cat. No.: MUXES-90011) to neutralize trypsin. Gently pipette the suspension up and down to detach the cells from the culture surface.

   Note: **Pipet gently to avoid generating bubbles. For routine passaging, mESCs do not need to be dissociated into a single-cell suspension. Instead, disaggregate them into small clusters of 2–3 cells.**

6. Centrifuge the cell suspension at 250×g for 5 minutes and aspirate the supernatant.
7. Resuspend the cell pellet in 2–3 mL of Serum Free Medium Type II (Cat. No.: MUXES-90061).
8. Seed the cells into gelatin-coated culture vessels at a density of (1~2)×$10^4$ viable cells/ $cm^2$.
9. Add an appropriate volume of Serum Free Medium Type II (Cat. No. MUXES-90061) and incubate at 37 °C with 5% $CO_2$ in a humidified incubator.