cyagen | OriCell™

User Manual

OriCell™ Serum Free Medium For Mouse Embryonic Stem Cells (Type I, With Feeder Layer)

Catalog No. MUXES-90062

We help you discover life

Introduction

OriCell™ Serum Free Medium For Mouse Embryonic Stem Cells (Type I, With Feeder Layer), carefully optimized by OriCell™ R&D team, contains a basal medium, selected serum-free supplements and others auxiliary ingredients.

This product is suitable for the serum-free culture of mouse embryonic stem cells (ES). It can maintain the good proliferation characteristics and highly undifferentiated totipotency of mouse embryonic stem cells in a serum-free and feeder-layer growth environment, and maintain correctness of the karyotype.

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

Components	Catalog Number	Volume
OriCell™ Basal Medium For Cell Culture	BKOD-03011	420 mL
OriCell™ Supplements For Mouse Embryonic Stem Cells Serum Free Culture (Type I)	MUXES-04062	5 mL
OriCell™ Serum Replacements For Mouse Embryonic Stem Cells Serum Free Culture (Type I)	MUXES-11062	75 mL

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 Handing Principles

1. Ensure that all equipment is kept clean and tidy.
2. Standard operation method. Please operate according to the method described in the product manual, strictly control the variables, and do a controlled experiment.
3. The ingredients should be properly stored in accordance with the storage conditions and used as soon as possible.
4. If a complete set of medium cannot be used in a short period of time, it should be prepared in batches according to the volume ratio of each component in the kit and stored in aliquots.

Product Stability and Storage Conditions

1. All ingredients must be kept in dark place.
2. The basal medium should be stored in a refrigerator at 4 °C for a period of 1 year. Other components should be stored at -20°C for a period of 2 years.

3. The prepared complete medium can be stored at 4°C for a period of 1 month. If the culture conditions are stable, the container has great sealing performance, and there is no alternation of hot and cold condition, the period of using can be appropriately extended, but not exceed 45 days.
4. Please use all products before the expiration date. Expired ingredients may significantly affect the cell culture effect.

Preparation of Complete Medium

Materials Required

- OriCell™ Serum Free Medium For Mouse Embryonic Stem Cells (Type I, With Feeder Layer) (Cat. No.: MUXES-90062)
- Clean, sterile and stable quality disposable consumables (pipettes, pipette tips, centrifuge tubes, etc.)
- Clean sealing film
- Aluminum foil paper and other light-avoiding materials

Steps

1. At least 6 hours before preparation, place the OriCell™ Serum Replacements (Cat. No.: MUXES-11062) in a 4°C refrigerator to completely thaw.
2. At least 30 minutes before preparation, the OriCell™ Culture Supplements (Cat. No.: MUXES-04062) should be placed at room temperature until completely melted.
3. Use 75% ethanol to carefully wipe the outer packaging of all ingredients. Open the package in the clean bench.
4. Add all the serum replacements and supplements to OriCell™ Basal Medium (Cat. No.: BKOD-03011).
5. Tighten the cap of the basal medium bottle, shake gently and 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, but the remaining components must be stored in accordance with their respective storage conditions and not be frozen and thawed multiple times.

2) Please choose whether to add antibiotics (e.g.: Penicillin/Streptomycin) according to your own needs. If you do need, please purchase them yourself.

6. Seal the bottle opening with parafilm, wrap the bottle in aluminum foil, 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 I, With Feeder Layer) 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: In order to make mouse embryonic stem cells grow better under serum-free conditions, it is strongly recommended to coat the surface of the culture vessel with gelatin.

1. Add enough 0.1% gelatin solution to cover the entire bottom of the culture vessel.
2. Leave at room temperature for at least 30 minutes.
3. If it is not used immediately, it can be sealed with a parafilm and stored at 4°C. Please use it within a week.
4. Before use, suck off (remove) the gelatin solution and dry it for later use.

Conversion 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™ Complete Medium For Mouse Embryonic Stem Cells (Cat. No.: MUXES-90011)

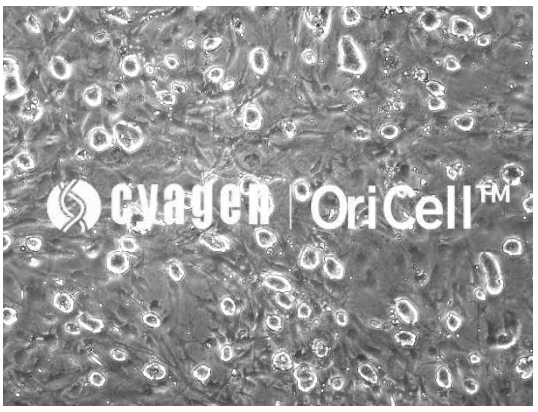
Steps (Feeder Layer with Serum → Feeder Layer without Serum)

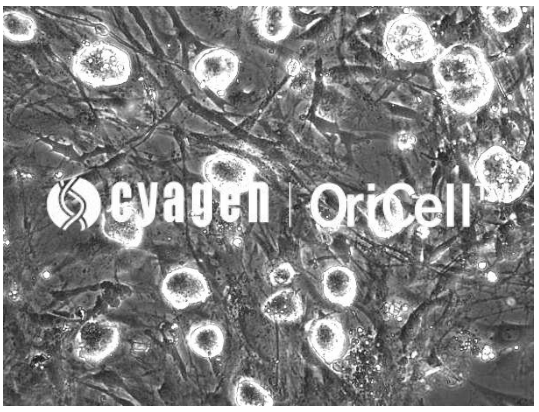
1. Resuscitate the feeder layer (Feeder) according to the conventional method, inoculate the feeder cells into the gelatin-coated culture vessel at 2.5×10^4 cells/cm^2 of living cells, and use it for the experiment after 24 hours.
2. Resuscitate mouse embryonic stem cells according to conventional methods, or directly digest mouse embryonic stem cells cultured in serum and feeder layers.
3. Collect the cells by centrifugation at 250×g for 5 min.
4. Resuspend mouse embryonic stem cells in the original serum medium, inoculate all of them into a culture vessel coated with gelatin, and place them in a 37°C, 5% CO_2, saturated humidity incubator for 30-40 min. It is possible to remove Feeder cells in the original cell population.

5. After 30-40 minutes (the specific time is judged by the degree of adhesion of the feeder cells), collect the supernatant medium, and centrifuge at 250×g for 5 minutes.
6. Aspirate the supernatant medium, and resuspend the cells with mouse embryonic stem cell type I serum-free complete medium. Inoculate in a prepared Feeder cell culture vessel according to the ratio required for the experiment.
7. Add a sufficient amount of mouse embryonic stem cell type I serum-free complete medium, and place it in a 37°C, 5% CO^2 incubator with saturated humidity.
8. The next day, observe the mouse embryonic stem cells cultured in the mouse embryonic stem cell type I serum-free complete medium for the first time, and replace the fresh mouse embryonic stem cell type I complete medium to remove the residual feeder cells and ES cells that died due to inability to adapt to serum-free conditions.

C57BL/6 Mouse Embryonic Stem Cells Were Cultured For 2 Days

With Feeder Layer and Serum-free





Passaging in Serum-Free 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™ Complete Medium For Mouse Embryonic Stem Cells (Cat. No.: MUXES-90011)
- OriCell™ Serum Free Medium For Mouse Embryonic Stem Cells (Type I, With Feeder Layer) (Cat. No.: MUXES-90062)

Steps

1. Warm mouse embryonic stem cell type I serum-free complete medium, 1×PBS, trypsin to 37°C.
2. Aspirate the used medium from the mouse embryonic stem cell culture vessel.
3. Wash 2~3 times with 1×PBS to remove residual medium.
4. Add trypsin (approximately 1 mL in a 35 mm diameter petri dish, and about 2~3 mL in a 100 mm diameter petri dish). Rotate gently to cover the cell surface with trypsin and digest until the mouse embryonic stem cells are separated.

   Note: **Due to the different titer of trypsin used in different laboratories, the digestion time may be slightly different. The specific time should be based on the observation under the microscope.**

5. Add 2 mL or more of mouse embryonic stem cell complete medium (containing serum), suck the liquid with a pipette, and blow the cells along the edge of the vessel, and the cells can be seen to fall off in a membranous state. When the membrane is sucked into the pipette tip and repeatedly blown several times, the membrane will gradually become thinner, and the ES cells will fall off from the membrane during the process of pipetting. Subsequently, the membrane was aspirated, and the remaining cell suspension was transferred to a centrifuge tube.

   Note: **1) Due to the characteristics of the culture system, the feeder layer will form a relatively**

dense membrane. During the digestion process, a relatively complete membrane can be formed and then peeled off. The feeder layer can be separated from the embryonic stem cells by gentle pipetting.

2） Pay attention to control the time during digestion to prevent the feeder membrane from rupturing and dispersing due to excessive digestion. The action should be gentle when blowing to prevent air bubbles.

3） The mouse embryonic stem cells do not need to be digested into a single cell suspension for passage, but can be digested into a single cell or 2 to 3 cell clusters.

6. Centrifuge the cell suspension obtained in the previous step at 250×g for 5 min, and aspirate the supernatant.
7. Resuspend the cells with 2~3 mL mouse embryonic stem cell type I serum-free complete medium.
8. According to (1~2)×10^4 viable cells/cm^2, inoculate into the culture vessel prepared with feeder in advance.
9. Add enough mouse embryonic stem cell type I serum-free complete medium, and place it in a 37°C, 5% CO_2 incubator with saturated humidity.