cyagen | OriCell

User Manual

OriCell™ Embryoid Body Formation Medium For Mouse Embryonic Stem Cells

Catalog No. MUXES-90051

We help you discover life

Introduction

Embryonic stem cells (ESCs) are totipotent stem cells derived from the inner cell mass of the blastocyst. They have the ability to differentiate into ectoderm, endoderm, and mesoderm, and can develop into various cell types. Unlike other stem cells, embryonic stem cells possess unlimited proliferative capacity. The plasticity and unlimited proliferation ability of embryonic stem cells make them a research hotspot in regenerative medicine and tissue engineering.

The formation of embryoid body (EB) is a key step in the differentiation of embryonic stem cells. In the absence of mouse embryonic fibroblast (MEF) feeder layers, embryonic stem cells spontaneously differentiate to form three-dimensional aggregates under the stimulation of EB formation medium. This structure facilitates cell interactions, such as cell-cell contact and gap junction formation.

The OriCell™ research team has carefully developed the OriCell™ Embryoid Body Formation Medium For Mouse Embryonic Stem Cells, which includes the EB formation basal medium, OriCell™ Fetal Bovine Serum (Superior-Quality), and other additives necessary for cell growth.

OriCell™ Embryoid Body Formation Medium For Mouse Embryonic Stem Cells can enhance the ability of mouse embryonic stem cells (ESCs) to differentiate and form EB. This product can be used to culture EB either by the hanging drop method or in non-adhesive petri dishes for suspension 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	BHDM-03011	447 mL	4 °C
OriCell™ Fetal Bovine Serum (Superior-Quality)	FBSSR-01021	50 mL	-20 °C
OriCell™ Supplements For Mouse Embryoid Body Formation	MUXES-04051	3 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 reference "COA" for details.

General Handing 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 component has 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™ Embryoid Body Formation Medium For Mouse Embryonic Stem Cells (Cat. No.: MUXES-90051)
- 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 6 hours before preparation, place the OriCell™ Fetal Bovine Serum (Superior-Quality) (Cat. No.: FBSSR-01021) in a refrigerator at 4 °C to allow it to thaw completely.

   Note: **Flocs (primarily composed of fibrin) may appear in thawed serum and will not affect product performance. Removal of flocs is generally unnecessary unless the cell culture system demands a high degree of purification.**

2. At least 30 minutes before preparation, place the OriCell™ Supplements For Mouse Embryoid Body Formation (Cat. No.: MUXES-04051) at room temperature until completely thawed.
3. Carefully wipe the outer packaging of all components with 75% ethanol. Open the package inside a clean bench (laminar flow hood).
4. Add all of the serum (Cat. No.: FBSSR-01021) and supplements (Cat. No.: MUXES-04051) to OriCell™ Basal Medium For Cell Culture (Cat. No.: BHDM-03011).
5. 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.

6. 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 the OriCell™ Embryoid Body Formation Medium For Mouse Embryonic**

Stem Cells are strictly aseptically controlled. Under normal circumstances, we do not recommend sterilizing it again. However, if there is a risk of contamination during the preparation process, the complete medium can be filtered and sterilized.

The Formation of Embryoid Body

Materials Required

- OriCell™ Embryoid Body Formation Medium For Mouse Embryonic Stem Cells (Cat. No.: MUXES-90051)

Steps

1. Prepare a 100 mm cell culture dish coated with gelatin.
2. Prepare embryonic stem cells (approximately 1×10^7 cells) in a T75 flask. Embryoid body (EB) formation can be initiated when the cells reach the logarithmic growth phase.
3. Digest the cells. Note: Digest the cells into single cells to ensure uniformity. Add embryoid body formation medium to stop the digestion.
4. Collect the cells and transfer the cell suspension into a 15 mL centrifuge tube. Centrifuge at 250 × g for 5 minutes.
5. Carefully discard the supernatant.
6. Resuspend the cells in embryoid body formation medium and seed approximately 5×10^6 cells into the gelatin-coated dish, adding about 8 mL of embryoid body formation medium.
7. Place the dish in an incubator at 37 °C, with 5% CO_2 and saturated humidity for 40 minutes to allow adhesion and remove mouse embryonic fibroblasts (MEF).
8. Remove the dish and gently collect the non-adherent cells (mostly embryonic stem cells) for the next step. If many MEF cells remain in the suspension, repeat steps 5 to 8 once more to further

remove MEF by adhesion.

9. Count the cells and adjust the cell density to 5.5 × 10^4 viable cells/mL. Seed the cells into 60 mm cell culture dishes, adding 5 mL of cell suspension per dish.

   Note: **Use non-tissue culture treated (non-TC treated) vessels suitable for suspension culture at this step.**

10. Incubate the dishes in an incubator at 37 °C, with 5% CO_2 and saturated humidity for 48 hours.
11. After two days, spherical suspended embryoid bodies of uneven sizes can be observed. Most embryoid bodies are small, translucent, and show good refractivity. Perform medium change by centrifugation at 140 × g for 1 minute.
12. After medium change, seed the embryoid bodies into new non-TC treated dishes and continue culture in the incubator at 37°C, 5% CO_2, saturated humidity for another 72 hours.
13. Over the next three days, the embryoid bodies gradually enlarge. Under high magnification, embryoid bodies should appear translucent and compact, with some showing aggregation or adhesion tendencies.
14. After 5 days of suspension culture, centrifuge at 140 × g for 1 minute. Resuspend the embryoid bodies in embryoid body formation medium and seed them into 24-well plates at 1 mL per well, with about 10–20 embryoid bodies per well, totaling 8 wells.
15. Continue culture in the incubator at 37 ° C, 5% CO_2, saturated humidity for 14 days, changing medium every 2–3 days. Observe the differentiation of embryoid bodies. If differentiation is ideal, spontaneous beating of differentiated cardiomyocytes can be observed around days 5 – 7 of differentiation.
16. After 14 days, use immunofluorescence to detect differentiation into endoderm, mesoderm, and ectoderm lineages.

Image of Embryoid Body (EB)