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User Manual

OriCell™ Neuronal Differentiation Medium For Mouse Embryonic Stem Cells

Catalog No. MUXES-90081

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.

Neural regeneration holds great promise for patients suffering from neurodegenerative diseases or injuries, such as Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease, and spinal cord injuries. However, expanding functional neurons for transplantation and scientific research remains a major challenge.

OriCell™ Neuronal Differentiation Medium For Mouse Embryonic Stem Cells effectively induces the differentiation of mouse embryonic stem cells into neurons. These differentiated cells are ideally suited for a wide range of downstream biological experiments.

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 of Solution A	Catalog Number	Volume	Storage
OriCell™ Basal Medium For Cell Culture A	BHDM-03011	178 mL	4 °C
OriCell™ Fetal Bovine Serum (Superior-Quality)	FBSSR-01021	20 mL	-20 °C
OriCell™ Supplements For Mouse ES Neuronal Differentiation A	MUXES-04081-a	2 mL	-20 °C

Components of Solution B	Catalog Number	Volume	Storage
OriCell™ Basal Medium For Cell Culture B	BNRO-03011	98 mL	4 °C
OriCell™ Supplements For Mouse ES Neuronal Differentiation B	MUXES-04081-b	2 mL	-20 °C

Other Components	Catalog Number	Volume	Storage
Poly-L-Lysine Solution	PLLY-10001	200 μL	-20 °C
Gelatin	GLT-11301	50 mL	4 °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 and gelatin have a shelf life of 1 year and should be stored at 4 °C. Fetal bovine serum, Supplement A, and Supplement B have a shelf life of 2 years and should be stored at −20 °C. The remaining component (Poly-L-Lysine Solution) has a shelf life of 1 year 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 ™ Neuronal Differentiation Medium For Mouse Embryonic Stem Cells (Cat. No.: MUXES-90081)
- Clean, sterile, and stable quality disposable consumables (pipettes, pipette tips, centrifuge tubes, etc.)
- Clean sealing film
- Aluminum foil and other light-avoiding materials

Steps

Preparation of Solution A

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 ES Neuronal Differentiation A (Cat. No.: MUXES-04081-a) in a refrigerator at 4 °C to allow it to thaw completely.
3. Mix the reagents by inverting the tube several times or gently flicking the bottom of the tube.
4. Carefully wipe the outer packaging of all components with 75% ethanol. Open the package inside a clean bench (laminar flow hood).
5. Add serum (Cat. No.: FBSSR-01021) and Supplement A (Cat. No.: MUXES-04081-a) to Basal Medium A (Cat. No.: BHDM-03011).
6. Rinse each bottle and tube with a small volume of Basal Medium A, then transfer the washings back

to the basal medium bottle to maximize recovery.

7. Securely tighten the cap on the basal medium bottle. Mix thoroughly by gentle swirling or inversion.

   Note: **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.**

8. 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™ Neuronal Differentiation 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.**

Preparation of Solution B

1. At least 30 minutes before preparation, place the OriCell™ Supplements For Mouse ES Neuronal Differentiation B (Cat. No.: MUXES-04081-b) in a refrigerator at 4 °C to allow it to thaw completely.
2. Mix the reagents by inverting the tube several times or gently flicking the bottom of the tube.
3. Carefully wipe the outer packaging of all components with 75% ethanol. Open the package inside a clean bench (laminar flow hood).
4. Add Supplement B (Cat. No.: MUXES-04081-b) to Basal Medium B (Cat. No.: BNRO-03011).
5. Rinse each bottle and tube with a small volume of Basal Medium A, then transfer the washings back to the basal medium bottle to maximize recovery.
6. Securely tighten the cap on the basal medium bottle. Mix thoroughly by gentle swirling or inversion.

   Note: **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.

7. 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™ Neuronal Differentiation 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.

Cell Culture Protocol

Materials Required

- OriCell™ Complete Medium For Mouse Embryonic Stem Cells (Cat. No.: MUXES-90011)
- OriCell™ 0.1% Gelatin Solution (Cat. No.: GLT-11301)
- OriCell™ 0.25% Trypsin-0.04% EDTA Solution (Cat. No.: TEDTA-10001)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)

Steps

Coat the Surface of the Culture Vessel with 0.1% Gelatin Solution

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.**

Mouse Embryonic Stem Cell Culture Protocol

1. Pre-warm the complete medium, PBS and trypsin to 37 °C.
2. Digest mouse embryonic stem cells with trypsin at 37 °C for approximately 1–2 minutes.
3. Add an appropriate amount of complete medium (2 mL per well for a 6-well plate) to stop the digestion, then gently pipette to resuspend the cells.

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

4. Transfer the cell suspension to a 15 mL centrifuge tube. Centrifuge all collected cell suspensions at 250 × g for 4 minutes.
5. Remove the supernatant after centrifugation. Add 5 mL of complete medium (Cat. No.: MUXES-90011), gently resuspend the cell pellet by pipetting up and down to thoroughly mix.
6. Seed the cells into a gelatin-coated 10 cm culture dish containing 7 mL of complete medium. Gently rock the dish to ensure even cell distribution and incubate for 30–40 minutes to allow cell attachment.

   Note: **Compared to undifferentiated embryonic stem cells (ESCs), mouse embryonic fibroblasts (MEFs) adhere more rapidly to the culture surface, allowing the ESCs to remain in suspension and enabling easy separation after a brief incubation.**

7. Collect the culture medium from the dish into a 15 mL centrifuge tube and centrifuge the collected

cell suspension at 250 × g for 4 minutes.

8. Carefully remove the supernatant after centrifugation. Add 2 mL of complete medium and gently resuspend the cell pellet by pipetting up and down to thoroughly mix.
9. Perform cell counting and prepare a cell suspension at a concentration of 1 – 2 × 10^5 cells/mL.
10. Add 10 mL of the prepared cell suspension to an untreated 10 cm culture dish.
11. Gently swirl the dish to mix the cells, then place it in a humidified incubator at 37°C with 5% CO_2. Incubate the cells for 2 days.
12. After 2 days, transfer the culture medium from the dish into a 50 mL centrifuge tube and allow the cells to settle at room temperature for approximately 15 minutes.
13. Carefully aspirate the supernatant, resuspend the cells in 10 mL of complete medium, and seed them into an untreated 10 cm culture dish.
14. Gently mix the cells and incubate in a humidified incubator at 37 °C with 5% CO_2 for an additional 2 days.

Procedure for Inducing Differentiation

Materials Required

- OriCell™ Neuronal Differentiation Medium For Mouse Embryonic Stem Cells (Cat. No.: MUXES-90081)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)
- OriCell™ Poly-L-lysine Solution (Cat. No.: PLLY-10001)
- Laminin
- Sterile Water for Injection

Steps

Coat the Surface of the Culture Vessel with Poly-L-lysine (PLL) and Laminin

1. Prepare the PLL/Laminin coating at least one day before the cell differentiation experiment.
2. Dilute Poly-L-lysine (PLL) with sterile ultrapure water to a final concentration of 15 μg/mL.
3. Add an appropriate volume of the diluted PLL solution to each well, ensuring the bottom surface is completely covered.
4. Incubate at room temperature for 30 minutes.
5. Aspirate the PLL solution and wash once with sterile water.
6. Dilute Laminin with sterile ultrapure water to a final concentration of 15 μg/mL.
7. Add the diluted Laminin solution to the culture vessels, ensure even coverage, seal with Parafilm, and incubate overnight at 4 °C.

   Note: **Coated plates can be stored at 4 °C for up to one week under sterile conditions. Do not allow the coating to dry out.**

8. Before use, aspirate the Laminin solution and rinse once with PBS. Allow the surface to air-dry before seeding cells.

Neuronal Cell Induction and Differentiation Protocol

1. After 4 days of cell culture, collect the cells into a 50 mL centrifuge tube.
2. Let the tube stand at room temperature for approximately 15 minutes to allow the cells to settle naturally to the bottom.
3. Aspirate the supernatant, resuspend the cells in 10 mL of Solution A, and seed into an untreated 10 cm culture dish.
4. Gently mix the cells and incubate in a humidified incubator at 37 °C with 5% CO_2 for 2 days.
5. After 2 days, repeat steps 1–4.
6. Gently mix the cells and incubate for an additional 2 days under the same conditions.
7. Gently swirl the dish to cluster the cells in the center. Under the microscope, approximately 50–100

cell clusters should be visible per dish.

8. Collect the culture medium from the dish into a 50 mL centrifuge tube.
9. Allow the cells to settle at room temperature for approximately 15 minutes.
10. Aspirate the supernatant and resuspend the cells in 10 mL of Solution B.
11. Seed 10–20 cell clusters into one well of a 24-well plate pre-coated with PLL/Laminin.
12. Gently mix the cells and place the plate in a humidified incubator at 37 °C with 5% CO_2. Continue culturing the cells for 2 days.
13. Afterward, perform a complete medium change with fresh Solution B every 2 days until day 6. Neuronal adherence and differentiation typically become visible by day 3 of culture.

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