OriCell Mouse Embryonic Stem Cell

Neurogenic Differentiation Medium

Catalog No. MUXES-90081

Product Description:

Neural cell regeneration has the potential to benefit patients who have nervous system degeneration problem or injuries, such as Parkinson`s disease (PD), Alzheimer`s disease (AZ), Huntington`s disease or spinal cord injuries, but the expansion of functional neurons for transplantion and scientific research remains difficult.

Embryonic stem cells (ESCs) are pluripotent cells derived from the inner cell mass of blastocyst-stage embryos and can be propagated infinitely in an undifferentiated state. They have the unique ability to differentiate into cells comprising all three embryonic germ layers (ectoderm, mouse ESCoderm and endoderm). Studies have led to the development of appropriate culture conditions and protocols for the generation of a broad spectrum of lineages. The ability to derive multiple lineages from ES cells opens exciting new opportunities to model embryonic development in vitro for studying the events regulating the earlist stages of lineage induction and specification.

OriCell Mouse Embryonic Stem Cell Neurogenic Differentiation Kit (Cat No. MUXES-90081) provides a system designed for the optimal differentiation of mouse ESCs into neurons. The kit provides enough reagents for ten separate differentiation reactions.

The product is intended for laboratory research use only, not for drug, house hold, or other uses.

Kit Component:

Mouse Embryonic Stem Cell Neurogenic Differentiation Medium A:

Mouse Embryonic Stem Cell Neurogenic Differentiation Basal Medium A	174 mL
(Cat. No. MUXES-03081)	
Mouse Embryonic Stem Cell- Qualified Fetal Bovine Serum	20 mL
(Cat. No. MUXES-05001)	
Penincillin-Streptomycin	2 mL
Glutamine	2 mL
Nonessential Amino Acid	2 mL

2-Mercaptoethanol	200 µL
Retinoic Acid Solution	40 µL
Mouse Embryonic Stem Cell Neurogenic Differentiation Medium B:	
Mouse Embryonic Stem Cell Neurogenic Differentiation Basal Medium B	97mL
(Cat. No. MUXES-03082)	
Neurogenic Differentiation Supplement	2 mL
(Cat. No. MUXES-04081)	
Glutamax Solution	1 mL
0.1% Gelatin Solution	**50 mL**
Poly-L-lysine	**200 µL**

Instructions:

Gelatin Coating of Tissue Culture Vessels

1. Add sufficient 0.1% Gelatin Solution into the culture vessel to completely cover its base.
2. Swirl until Gelatin Solution coats entire base of vessel. Let sit for at least 30 minutes at room temperature.
3. Aspirate off all of the Gelatin Solution and allow the remainder to evaporate by leaving the vessel sitting open in the hood for no more than 30 minutes.
4. Put lid back once the surface is dry.

Formation of Embryoid Bodies

1. Dissociate mouse ESCs by incubating the cells with trypsin solution at 37°C for 1-2 min.
2. Add an appropriate volume of Embryoid Body (EB) Formation Medium (Cat. No. MUXES-90051) (e.g. 2 mL for each well of six-well plate) to stop reaction and gently pipette up and down until cells in colonies become single cells.
3. Transfer cell suspension into a 15 mL conical tube and centrifuge at 250 g for 5 minutes to pellet the cells.

4. Carefully aspirate as much of the supernatant as possible.
5. Apply 5 mL Embryoid Body (EB) Formation Medium (pre-warm to 37°C) to the centrifuge tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex.
6. Transfer the cell suspension to a fresh sterile 100 mm plate that has been pre-coated with 0.1% gelatin solution. Apply 7mL Embryoid Body (EB) Formation Medium to the 100 mm plate。
7. Incubate the adherent dishes in 37°C incubator for 30~40 minutes to separate MEFs from ESCs.
8. Carefully collect the suspending cells into 15 mL polypropylene culture tubes.
9. Centrifuge the cells at 250 g for 4 minutes at room temperature.

Note: Mouse embryonic fibroblasts (MEF) adhere more readily to the tissue-culture plate than undifferentiated mouse ES cells and thus a significant number of MEF can be removed by this differential adhesion step.

10. Discard the supernatant. Apply 2 mL EB Formation Medium to the conical tube and resuspend the cells thoroughly.

    IMPORTANT: Do not vortex.
11. Count the number of cells and adjust the cell concentration to $1\sim2\times10^5$ cells/mL with EB formation medium.
12. Plate 10 mL cell suspension in a sterile 100 mm non-adherent Petri dish.
13. Incubate the cells in 37°C, 5% CO_2 incubator for two days. After two days, there should be numerous floating aggregates formed.
14. On the second day, transfer the floating EBs and the entire medium in the 100 mm Petri dish to a 50 mL conical tube.
15. Leave the conical tube at room temperature for 15 minutes to allow the EBs to settle to the bottom of the tube.
16. Carefully remove the supernatant. Resuspend the EBs in 10 mL EB Formation Medium (pre-warmed to 37°C).
17. Transfer the entire cell suspension to a sterile 100mm non-adherent Petri dish. Incubate the EBs in 37°C, 5% CO_2 incubator for an additional two days.

Neurogenic Protocol

Preparation of complete medium

Prior to use, thaw mouse ESC Neurogenic Differentiation Basal Medium A, mouse ESC Neurogenic Differentiation Basal Medium B, and other supplements at room temperature. Gently swirl the vial.

Note: Centrifuge the vials briefly at low speed before removing the caps to ensure recovery of entire content.

Preparation of mouse ESC Neurogenic Differentiation Medium A

1. Transfer the entire amount of Fetal Bovine Serum, Penincillin-Streptomycin, Glutamine, Nonessential Amino Acid, 2-Mercaptoethanol and Retinoic Acid Solution into mouse ESC Neurogenic Differentiation Basal Medium A.
2. Rinse each vial with the medium and transfer the rinse medium back to the bottle of basal medium as much as possible.
3. Repeat step 2 several times.
4. Gently swirl the fully supplemented complete medium to ensure a homogeneous mixture. The mouse ESC Neurogenic Differentiation Medium A is now ready to use.

Note: The Retinoic Acid Solution A is sensitive to light, it can be stored in dark at 2-8 °C for just one week. For a long period storage, it should be kept at -20 °C.

Preparation of mouse ESC Neurogenic Differentiation Medium B

1. Transfer the entire amount of Glutamax solution and Neurogenic Differentiation Supplements into Neurogenic Differentiation basal Solution B.
2. Rinse each vial with the medium and transfer the rinse medium back to the bottle of basal medium as much as possible.
3. Repeat step 2 several times.
4. Gently swirl the fully supplemented complete medium to ensure a homogeneous mixture. The mouse ESC Neurogenic Differentiation Medium B is now ready to use.

Note: Although each component in this kit is supplied sterile, it is strongly recommended to filter the mouse ESC Neurogenic Differentiation Medium B.

Induction of Neuronal Differentiation

1. After a total of 4 days in suspension culture, collect the EBs to a 50 mL conical tube.
2. Leave the conical tube at room temperature for 15 minutes to allow the EBs to settle to the bottom of the tube.

3. To the tube containing the EBs, carefully remove and discard the supernatant with a 10 mL pipette .
4. Resuspend the EBs in 10 mL mouse ESC Neurogenic Differentiation Medium A and transfer the suspension to a fresh non-adherent Petri dish.
5. Incubate the dish of cells in 37°C, 5% CO_2 incubator for two days.
6. After two days, repeat steps 1 through 6, Exchange the fresh mouse ESC Neurogenic Differentiation Medium A.
7. Incubate the dish of cells in 37°C, 5% CO_2 incubator for an additional two days.

Preparation of Coated Tissue Culture Vessels for expansion

1. Dilute Poly-L-lysine stock Solution (1 mg/mL) with water to yield 15 µg/mL solution.
2. Add enough of Poly-L-lysine Solution into the culture vessel to completely cover its base.
3. Swirl until Poly-L-lysine Solution coats entire base of vessel. Let sit for at least 30 minutes at room temperature.
4. Aspirate off all of the Poly-L-lysine Solution and rinse the vessel once with sterile water. Aspirate after each rinse.
5. Using sterile 1× PBS, dilute the Laminin stock Solution (1 mg/mL) to a final concentration of 15 µg/mL.
6. Add enough of Laminin Solution into the culture vessel to completely cover its base. Incubate overnight at 4 °C.
7. Coated vessels can be stored in the Laminin Solution at 4°C for one week.
8. Just before use, aspirate the laminin solution in the coated vessels and wash the wells once with 1× PBS. Aspirate after rinse.

Expansion of Neuronal Cells from Embryoid Bodies

1. From step 7 of section titled “Induction of Neuronal Differentiation, gently swirl the 100 mm non-adherent Petri dish containing the neural induced EBs counterclockwise for several circular rotations.

Note: Use caution to avoid sloshing the media over the side of the plate as this may increase the risk of cell contamination. The swirling process helps localize all of the EBs to the center of the 100mm Petri dish.

2. Immediately place the 100mm Petri dish under a microscope. This is your total number of EBs in the culture. Typical yields are 50~100 EBs per 100mm Petri dish.
3. Collect and transfer the EB suspension to a 50 mL conical tube.
4. Leave the conical tube at room temperature for 15 minutes to allow the EBs to settle to the bottom of the tube.

5. Remove and discard the supernatant.
6. Resuspend the EBs in 10 mL mouse ESC Neurogenic Differentiation Medium B (pre-warmed to 37°C).
7. Plate approximately 10~20 EBs to each well of a Poly-L-lysine/Laminin coated 24-well Tissue Culture Plate (or other Tissue Culture Plate).
8. Incubate the dish of cells in 37°C, 5% CO_2 incubator for two days.
9. Exchange the medium in each well with 2 mL fresh mouse ESC Neurogenic Differentiation Medium B every two days for a total of 6 days. Neuronal like cells and their processes should extend and migrate from the attached EBs in as little as three days.

Note: Use extreme care when exchanging the medium as the cells are loosely adherent. Always leave behind a small volume of medium to ensure that the cells do not dry out.

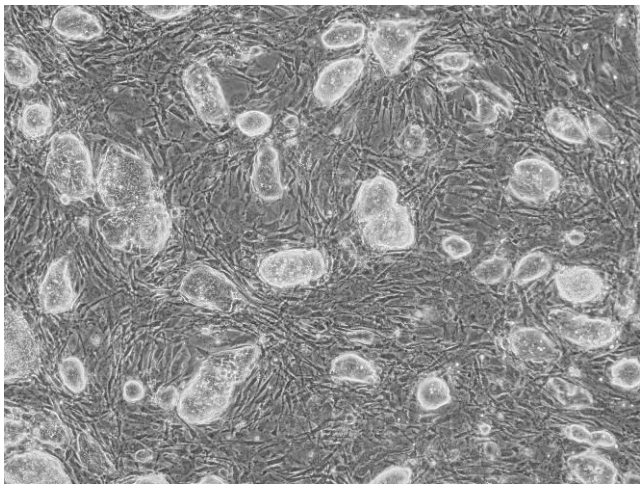

Figure 1. Undifferentiated mouse ES cells display the characteristic tight round colonies with a high nuclear to cytoplasmic ratio. (magnification x 100).

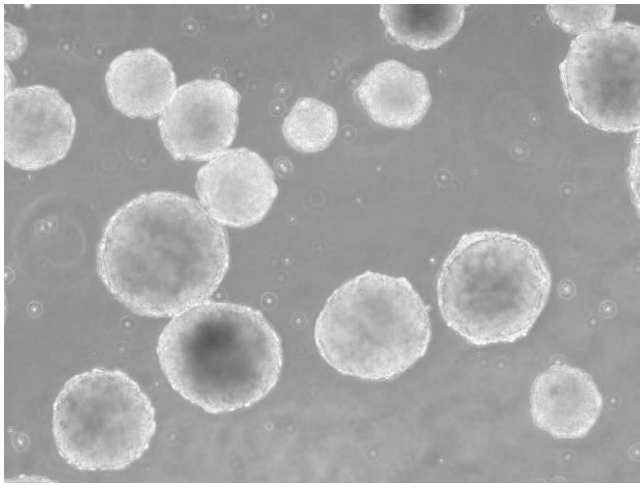

Figure 2. Formation of embryoid bodies (EB) after the culture of dissociated mouse ES cells in EB Formation Medium for 2 days on a non-adhesive Petri dish. (magnification x 100).

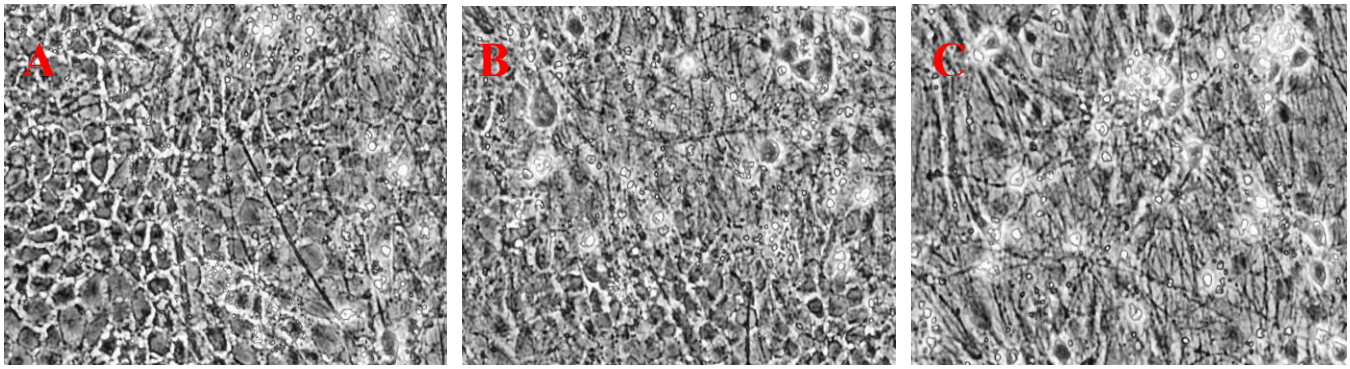

Figure 3. Differentiation of mouse ES cells to neurons after treatment with Mouse Embryonic Stem Cell (mouse ESC) Neurogenic Differentiation Medium B for 2 days(A), 4 days(B), and 6 days(C). (magnification x 200).

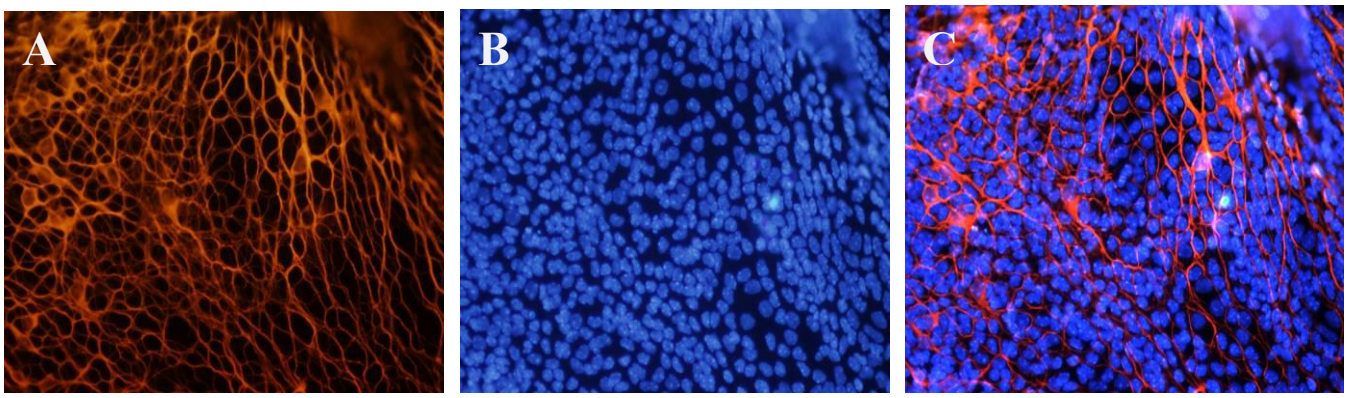

Figure 4. Immunofluorescent images of ES differentiated neurons. Stained with mouse anti β-Tubulin III(A), nuclei of the cells are visualized with Hochest(B), a merged image is presented (C).

Stability/Storage:

All products should be stored in the dark.

Neurogenic Differentiation Basal Medium and 0.1% Gelatin Solution is stable at 2 to 8 °C for up to one year. Other components are stable at -20 °C for up to two years. These products should be discarded beyond the labeled expiration date.

Once prepared, the fully supplemented complete medium can be stored for up to one month when stored in the dark at 2 to 8 °C. Retinoic Acid Solution is light sensitive and is readily oxidized upon exposure to air. Retinoic Acid Solution should stored for up to one week when stored in the dark at 2 to 8 °C. For a long period storage, it should be kept at -20 °C.

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For optimal performance, repeated warming/cooling and freeze-thawing should be avoided.

Quality Control:

Mouse Embryonic Stem Cell Neural Differentiation Kit is performance tested on Mouse Embryonic Stem Cells.

Standard evaluation includes:

1. Sterility test (bacteria, fungi, mold and mycoplasma)
2. pH test
3. Osmolality
4. Endotoxin