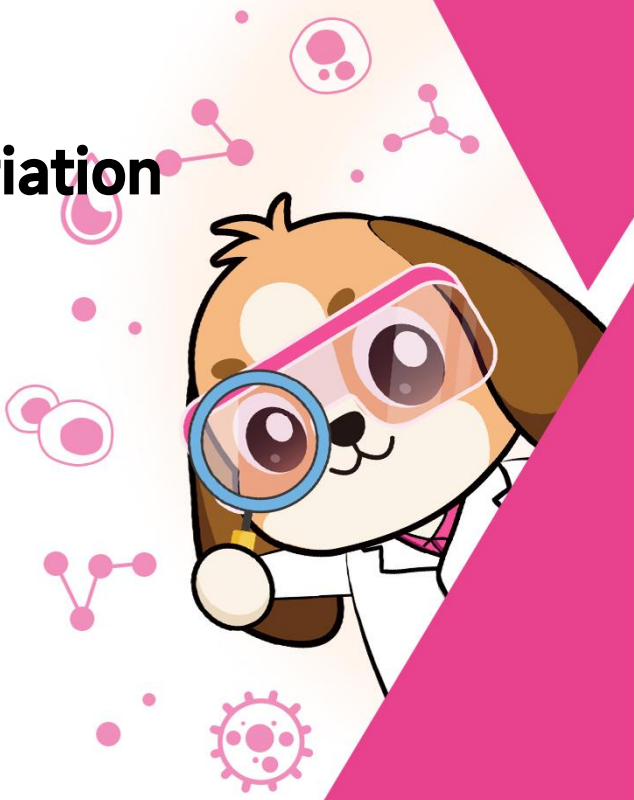
cyagen | OriCell™

User Manual

OriCell™ Neuronal Differentiation Medium For Mouse Neural Stem Cells



Catalog No. MUXNX-90081

We help you discover life

Introduction

Neurons are electrically excitable cells that process and transmit information through electrical and chemical signals. Chemical signaling occurs at specialized junctions called synapses. Neurons connect to form complex networks and serve as the core components of the nervous system, which includes the brain, spinal cord, and peripheral ganglia.

OriCell™ Neuronal Differentiation Medium For Mouse Neural Stem Cells contains a basal culture medium optimized for inducing neural stem cells to differentiate into neurons, along with essential additives. This product has been successfully used to promote the differentiation of mouse neural stem cells into neurons.

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	BNRO-03011	98 mL
OriCell™ Supplements For Mouse NSC Neuronal Differentiation	MUXNX-04081	2 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 within the expiration date. Expired ingredients may significantly affect the cell culture effect.

Preparation of Complete Medium

Materials Required

- OriCell™ Neuronal Differentiation Medium For Mouse Neural Stem Cells (Cat. No.: MUXNX-90081)
- 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 30 minutes before preparation, place the OriCell™ Supplements For Mouse NSC Neuronal Differentiation (Cat. No.: MUXNX-04081) at room temperature to thaw completely.
2. Invert or gently flick the reagent tube to mix the reagent.
3. Use 75% ethanol to carefully wipe the outer packaging of all ingredients. Open the package in the clean bench.
4. Add all of the supplement (Cat. No.: MUXNX-04081) to OriCell™ Basal Medium For Cell Culture(Cat. No.: BNRO-03011).
5. Tighten the cap of the basal medium bottle, shake gently and thoroughly.

   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.**

6. Seal the bottle opening with sealing film, wrap the bottle body with aluminum foil, and label it with the name, preparation date, and other relevant information.

Note: **All components in OriCell™ Neuronal Differentiation Medium For Mouse Neural Stem Cells 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.**

Procedure for Inducing Differentiation

Materials Required

- OriCell™ Neuronal Differentiation Medium For Mouse Neural Stem Cells (Cat. No.: MUXNX-90081)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)
- Poly-L-lysine（PLL）
- Laminin
- Sterile Ultrapure Water

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 and set aside.
3. Add an appropriate amount of the PLL solution from step 2 to the culture plate, gently shake to evenly cover the bottom surface.
4. Incubate at room temperature for 30 minutes.

5. Aspirate the PLL solution and wash once with an appropriate amount of sterile water.
6. Dilute Laminin with sterile ultrapure water to a final concentration of 15 μg/mL and set aside.
7. Add the Laminin solution from step 6 to the culture plate, gently shake to evenly cover the bottom surface, seal with sealing film, and incubate overnight at 4°C for later use.

   Note:

   The coated culture plates can be stored at 4°C for up to one week under sterile conditions without allowing the Laminin to dry out. Please use within one week.
8. Before use, aspirate the laminin and rinse once with PBS. Allow to air dry before use.

Protocol for Culturing Cells

1. Prewarm the complete medium to 37°C.
2. Prepare a 15 mL or other suitable centrifuge tube and collect the medium from the culture vessel.
3. Wash the cell culture vessels once with mouse nerve stem basal medium (if available), handling gently and thoroughly, then collect the basal medium.
4. Centrifuge all collected cell suspensions at 160×g for 5 minutes.
5. After centrifugation, remove the supernatant. Add 1 mL of complete medium and gently pipette the cell pellet about 15–20 times to fully resuspend and mix the cells.

   Note: **Pipetting should be gentle to avoid generating excessive bubbles, which may cause cell damage and loss.**
6. Seed the cells at a density of (2.5–4) × 10^4 viable cells/cm^2 into appropriate culture vessels.
7. Gently mix the cells and place them in a 37°C, 5% CO_2, saturated humidity incubator.
8. On the day after passaging, observe the cell condition and add fresh medium as needed.

   Note: **If you observe a significant amount of cell death after passaging, perform a medium change (refer to passaging steps 2–4: after centrifugation, remove the supernatant, gently resuspend the pellet a few times in 1 mL of complete medium, and then seed the cells; avoid repeated pipetting).**

Neuronal Cell Induction and Differentiation Protocol

1. Seed neural stem cells at a density of 2 × 10^4 cells/cm^2 into 6-well plates coated with PLL/Laminin
2. Gently mix the cells and place the plates in a 37°C, 5% CO_2, humidified incubator.
3. After 2 days, replace the medium with OriCell™ Neuronal Differentiation Medium For Mouse Neural Stem Cells to Neuron Induction Differentiation Medium.
4. Subsequently, change the induction differentiation medium every 3 days.
5. Perform relevant analyses or extract RNA/protein for downstream experiments on day 7.