OriCell Mouse Neural Stem Cell

Neurogenic Differentiation Medium

Catalog No. MUXNX-90081

Product Description:

A neuron is an electrically excitable cell that processes and transmits information by electrical and chemical signaling. Chemical signaling occurs via synapses, specialized connections with other cells. Neurons connect to each other to form networks. Neurons are the core components of the nervous system, which includes the brain, spinal cord, and peripheral ganglia.

Neural Stem Cell Neurogenic differentiation Medium consists of optimized Neural stem cell Neurogenic Differentiation Basal Medium and supplements. This product has been developed for the optimal differentiation of Mouse Neural Stem Cells (Cat. No.MUBNF-01001) into neurons.

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

Kit Components:

Neural Stem Cell Neurogenic Differentiation Basal Medium (Cat. No. MUXNX-03081)	97 mL
Neural Stem Cell Neurogenic Differentiation Supplement (Cat. No. MUXNX-04081)	2mL
Glutamax	1 mL

Instructions:

Preparation of Neural Stem Cell Neurogenic differentiation Medium

1. Prior to use, thaw Glutamax, Neural Stem Cell Neurogenic Differentiation Supplement solution at room temperature. Gently invert the vials to ensure homogeneity.

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

2. Disinfect with 70% v/v ethanol the external surfaces of the bottles/vials for every component in the

kit. Allow ethanol to evaporate away.

3. In a laminar flow hood aseptically open the bottles/vials.
4. Transfer the entire amount of Glutamax solution and Neural Stem Cell Neurogenic Differentiation Supplement into Neural stem cell Neurogenic differentiation Basal Medium.
5. Rinse each vial with the medium and transfer the rinse medium back to the bottle of basal medium as much as possible.
6. Repeat step 5 several times.
7. Gently swirl the fully supplemented complete medium to ensure a homogeneous mixture. The complete medium is now ready to use.

Note: Although each component in this kit is supplied sterile, it is strongly recommended to filter the fully supplemented complete medium.

Preparation of PLL/laminin Coated Tissue Culture Vessels

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

Mouse Neural Stem Cell Culture

1. Pre-warm Mouse Neural Stem Cell Growth Medium (Cat No. MUBNF-90081), 1×PBS, Trypsin-Like Enzyme solution to 37 °C.
2. Transfer the media containing the floating neurospheres to a 15 mL conical tube. Centrifuge at 250 g

for 5 minutes.

3. Aspirate and discard all the supernatant, add 2 mL of 1×PBS and 200 µL of Trypsin-Like Enzyme to the conical tube and re-suspend with a fire polished glass pipette.
4. Mechanically dissociate the neurospheres by gently pipetting up and down 8-10 times with a fire polished glass pipette, be careful not to introduce bubbles in the suspension.
5. Add 10 mL 1×PBS to the conical tube and mix well.
6. Centrifuge at 250 g for 5 minutes.
7. Aspirate as much of the supernatant as possible.
8. Re-suspend the cells in 3 mL of Mouse Neural Stem Cell Growth Medium (pre-warm to 37°C).
9. Plate cells into optimal tissue culture vessels and add sufficient Mouse Neural Stem Cell Growth Medium.
10. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.

Neurogenesis Protocol (for 6-well tissue culture plate)

1. For neurogenic differentiation, Neural stem cells are plated in 6-well tissue culture plates coated PLL/laminin at $2x10^4$ cells/cm^2 with growth medium volume of 2 mL per well.
2. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.
3. After two days, change the medium to Neural stem cell Neurogenic differentiation Medium by completely replacing the spent growth medium.
4. Replace fresh differentiation medium every 3 days.
5. Cells are collected for analysis or RNA/protein extraction at about 7 days.

Stability/Storage：

All products should be stored in the dark.

Neural stem cell Neurogenic differentiation Basal Medium 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 expimouseion 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.

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

Quality Control:

Neural stem cell Neurogenic differentiation Medium is performance tested on Neural stem cells.

Standard evaluation includes:

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