NEURAL STEM CELL BASAL MEDIUM

CATALOG NUMBER: SCM003 **QUANTITY:** 500 mL

LOT NUMBER:

DESCRIPTION:

The Neural Stem Cell Basal Medium is a defined serum-free, growth factor-free medium that has been optimized for the growth and *in vitro* differentiation of neural stem cells derived from rodents. When used in conjunction with FGF or FGF, EGF and heparin, the basal medium will allow for the proliferation of rat and mouse neural stem cells, respectively. Withdrawal of the growth factors from the Neural Stem Cell Basal Medium will result in the spontaneous differentiation of rodent neural stem cells.

PRESENTATION:

Serum-free formulation.
- Sterility Testing: Negative
- Osmolarity: 299
- PH: 7.4

MATERIALS REQUIRED BUT NOT SUPPLIED:

1. Cryopreserved rat hippocampal neural stem cells (Catalog No. SCR022)
2. Cryopreserved mouse cortical neural stem cells (Catalog No. SCR029)
3. Cryopreserved mouse spinal cord neural stem cells (Catalog No. SCR031)
4. Poly-L-ornithine (Sigma Catalog No. P3655)
5. Laminin (Catalog No. CC095)
6. Accutase™ Solution (Catalog No. SCR005)

RELATED PRODUCTS:

1. Neural Stem Cell Characterization Kit (Catalog No. SCR019)
2. Neural Stem Cell Freezing Medium (Catalog No. SCM014)

PREPARATION OF COATED PLATES:

We recommend coating tissue culture plastic- or glasswares that are used to culture rat/mouse neural stem cells with poly-L-ornithine and laminin. The following procedure is recommended:

1. Prepare stock solutions of poly-L-ornithine (10 mg/mL) by dissolving poly-L-ornithine in sterile water. The stock solution should be stored at -20°C or -80°C.
2. Dilute poly-L-ornithine with water from the stock concentration (10 mg/mL) to yield:
    a. 10 µg/mL for polystyrene plates
    b. 50 µg/mL for glass plates
3. Add enough of the poly-L-ornithine solution to cover the whole surface of the tissue culture-ware. Use 5 mL volume for 6-cm plates and 10 mL volume for 10-cm plates and T75 flasks. Incubate overnight at room temperature.
4. The next day, rinse the tissue culture-wares with sterile water. Aspirate after each rinse.
5. Using sterile 1X PBS, dilute laminin to a final concentration of 5-7 µg/mL. ***Note:*** *The same laminin concentration is used for both glass and polystyrene tissue culture-ware.*

6. Add enough laminin (5-7 µg/mL) solution to the tissue culture-ware to cover the surface. Use 5 mL volume for 6-cm plates and 10 mL volume for 10-cm plates and T75 flasks. Incubate overnight at room temperature.
7. Coated plates and flasks can be stored in the laminin solution at -20°C for 6-8 months. The plates should be wrapped in plastic saran wrap before storage at -20°C.
8. Just before use, aspirate the laminin solution in the coated plates and wash the plates once with 1X PBS.

THAWING CELLS:

1. Do not thaw the cells until the recommended medium and appropriately coated poly-L-ornithine and laminin plasticware and/or glassware are on hand.
2. Remove the vial of rat/mouse neural stem cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells. **IMPORTANT: Do not vortex the cells.**
3. As soon as the cells are completely thawed disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful to not introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of Neural Stem Cell Basal Medium (pre-warmed at 37°C) to the 15 mL conical tube. **IMPORTANT: Do not add the whole volume of medium at once to the cells. This may result in decreased cell viability due to osmotic shock.**
6. Gently mix the cell suspension by slow pipeting up and down twice. Be careful to not introduce any bubbles. **IMPORTANT: Do not vortex the cells.**
7. Centrifuge the tube at 300 xg for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 4-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in a total volume of 10 mL of Neural Stem Cell Basal Medium (pre-warmed to 37°C) containing freshly added growth factors.

   ***Note**: Growth factors should always be added fresh to the Neural Stem Cell Basal Medium. For rat neural stem cells: add 2 µL of FGF-2 (100 µg/mL stock) to 10 mL of Neural Stem Cell Basal Medium. For mouse neural stem cells, add 2 µL each of FGF (100 µg/mL stock), EGF (100 µg/mL stock) and heparin (10mg/mL stock) to 10 mL of Neural Stem Cell Basal Medium.*
10. Plate the cell mixture onto a poly-L-ornithine and laminin-coated 10-cm tissue culture plate.
11. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.
12. The next day, exchange the medium with fresh Neural Stem Cell Basal Medium (pre-warmed to 37°C) containing freshly added growth factors (refer to step 9 for growth factor concentrations). Exchange with fresh medium containing growth factors every other day thereafter.
13. When the cells are approximately 80% confluent, they can be dissociated with Accutase solution and passaged or alternatively frozen for later use.

SUBCULTURING:

1. Carefully remove the medium from the poly-L-ornithine and laminin-coated 10-cm tissue culture plate containing the confluent layer of rodent neural stem cells.
2. Apply 3-5 mL of Accutase solution and incubate in a 37°C incubator for 3 minutes.
3. Inspect the plate and ensure the complete detachment of cells by gently tapping the side of the plate with the palm of your hand.
4. Apply 5 mL of Neural Stem Cell Basal Medium (pre-warmed to 37°C) to the plate.
5. Transfer the dissociated cells to a 15 mL conical tube.
6. Centrifuge the tube at 300 xg for 2-3 minutes to pellet the cells.
7. Discard the supernatant
8. Apply 2 mL of Neural Stem Cell Basal Medium containing the appropriate concentrations of growth factors to the conical tube and resuspend the cells thoroughly.

   ***Note**: Growth factors should always be added fresh to the Neural Stem Cell Basal Medium. For rat neural stem cells: add 2 µL of FGF-2 (100 µg/mL stock) to 10 mL of Neural Stem Cell Basal Medium. For mouse neural stem cells, add 2 µL each of FGF (100 µg/mL stock), EGF (100 µg/mL stock) and heparin (10mg/mL stock) to 10 mL of Neural Stem Cell Basal Medium.*
9. Count the number of cells using a hemacytometer.
10. Plate the cells to the desired density into the appropriate poly-L-ornithine and laminin-coated flasks, plates or wells in Neural Stem Cell Basal Medium containing the appropriate concentrations of growth factors. We typically plate the cells at ~2 million cells on poly-L-ornithine and laminin coated 10-cm plates or T75 flasks.

STORAGE/HANDLING: Neural Stem Cell Basal Medium should be stored at -20°C until ready to use. Upon thawing, the basal medium may be stored at 2-8°C for up to one month.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

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