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MILLITRACE™ RAT NEURAL STEM CELL EXPANSION MEDIUM

CATALOG NUMBER: SCM040

LOT NUMBER:

QUANTITY: The following components are included:

500 mL Neural Stem Cell Basal Medium (Part No. SCM003)
5 mg/mL Puromycin Solution, 100 µL (Part No. CS201361)
10 µg Basic FGF, lyophilized (Part No. GF003-10UG)

DESCRIPTION: MilliTrace Rat Neural Stem Cell Expansion Medium is provided as a multi-component system that is convenient and easy to use. The Neural Stem Cell Basal Medium is a defined serum-free medium that has been optimized for the growth and *in vitro* differentiation of neural stem cells derived from rodents. Basic Fibroblast Growth Factor (FGF-2, bFGF) is provided separately, which when added to the Neural Stem Cell Basal Medium (SCM003) allows for the growth and proliferation of rat neural stem cells. Withdrawal of FGF-2 from the Neural Stem Cell Basal Medium will result in the spontaneous differentiation of rat neural stem cells. Puromycin Solution is provided separately to help maintain the expression of the GFP labeled transgene.

QUALITY CONTROL: Each lot of Neural Stem Cell Basal Medium is tested for:
Sterility Testing: Negative
Osmolarity: 299
PH: 7.4

MATERIALS REQUIRED BUT NOT SUPPLIED:

- MilliTrace Constitutive GFP Reporter Adult Rat Hippocampal Neural Stem Cell Kit (Millipore Catalog No. SCR080)
- Neural Stem Cell Characterization Kit (Millipore Catalog No. SCR019)
- Poly-L-ornithine (Sigma Catalog No. P3655)
- Laminin (Millipore Catalog No. CC095)
- Accutase™ (Millipore Catalog No. SCR005)

PREPARATION OF COATED PLATES: We recommend coating the tissue culture plastic- or glasswares that are used to culture rodent 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 entire surface of 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 OF CELLS:

Do not thaw the cells until the recommended medium and appropriately coated poly-L-ornithine and laminin plasticware and/or glassware are on hand.

1. Remove the vial of constitutive GFP reporter adult rat hippocampal neural stem cells (Part No. SCC080) 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.**

2. As soon as the cells are completely thawed disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.

3. 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.

4. 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.**

5. 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.**

6. Centrifuge the tube at 300 xg for 2-3 minutes to pellet the cells. Decant as much of the supernatant as possible. Steps 3-6 are necessary to remove residual cryopreservative (DMSO).

7. Resuspend the cells in a total volume of 10 mL of Neural Stem Cell Basal Medium (pre-warmed to 37℃) containing freshly added 20 ng/mL FGF-2 and 1 µg/mL puromycin.

   ***Note**: FGF-2 should always be added fresh to the Neural Stem Cell Basal Medium. To obtain final concentrations of 20 ng/mL FGF-2, add 2 µL of the FGF-2 stock solution to 10 mL of Neural Stem Cell Basal Medium. To help maintain expression of the GFP transgene, add puromycin to the Neural Stem Cell Basal Medium. To obtain final concentrations of 1 µg/mL puromycin, add 2 µL of the puromycin stock (5 mg/mL) to 10 mL of Neural Stem Cell Basal Medium*

8. Plate the cell mixture onto a poly-L-ornithine and laminin-coated 10-cm tissue culture plate. Incubate the cells at 37℃ in a 5% CO_2 humidified incubator.

9. The next day, exchange the medium with fresh Neural Stem Cell Basal Medium (pre-warmed to 37℃) containing 20 ng/mL FGF-2 and 1 µg/mL puromycin Exchange with fresh medium containing FGF-2 and puromycin every other day thereafter.

10. When the cells are approximately 80% confluent, they can be dissociated with Accutase 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 constitutive GFP reporter adult rat hippocampal neural stem cells.

2. Apply 3-5 mL of Accutase and incubate in a 37℃ 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℃) to the plate.

5. Transfer the dissociated cells to a 15 mL conical tube. Centrifuge the tube at 300 xg for 2-3 minutes to pellet the cells. Discard the supernatant.

6. Apply 2 mL of Neural Stem Cell Basal Medium containing 20 ng/mL FGF-2 and 1 µg/mL puromycin to the conical tube and resuspend the cells thoroughly.

7. Count the number of cells using a hemacytometer.

8. 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 20 ng/mL FGF-2 and 1 µg/mL puromycin. We typically plate the cells at ~2 million cells on poly-L-ornithine and laminin coated 10-cm plates or T75 flasks.

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STORAGE/HANDLING: Neural Stem Cell Basal Medium (Part No. SCM003) should be stored at -20°C until ready to use. Upon thawing, the basal medium should be stored at 2-8°C for up to one month.

Basic FGF, lyophilized (Part No. GF003-10UG) should be reconstituted with 100 µL 5 mM Tris-HCL, pH 7.6 for a final concentration of 100 µg/mL. Dispense into aliquots to avoid repeated freeze-thaw. Store at -20°C for up to six months. This solution can then be diluted into other aqueous buffers and stored at 4°C for 1 week or -20°C for future use. Multiple freeze/thaw cycles will result in significant loss of activity.

5 mg/mL Puromycin Solution, 100 µL (Part No. CS201361) should be stored in working aliquots at -20°C for up to two years.

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 as a diagnostic.

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

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