User Manual

Cryopreserved Mouse Cortical Neural Stem Cells

SCR029

Introduction

Neural stem cells (NSC) are self-renewing cells with the capacity to differentiate into neurons, astrocytes and oligodendrocytes. Due to their therapeutic promise, considerable attention has been focused on identifying the sources of stem cells, the signals that regulate their proliferation and the specification of neural stem cells towards more differentiated cell lineages.

Presently, neural stem cells are often identified based upon the presence of molecular markers that correlate with the stem and/or progenitor state along with the absence of differentiated phenotypes as assessed through marker analysis. To aid researchers in the accurate identification and characterization of neural stem cells, presents Cryopreserved Mouse Cortical Neural Stem Cells (SCR029).

Cryopreserved Mouse Cortical Neural Stem Cells provides ready-to use primary neural stem cells isolated from the cortices of embryonic day 15-18 (E15-E18) C57/BL6 mice. These primary neural stem cells may be used for a variety of research applications including drug development, for studies of neurotoxicity, neurogenesis, electrophysiology, neurotransmitter and receptor functions and CNS diseases and disorders.

We recommend that Cryopreserved Mouse Cortical Neural Stem Cells (SCR029) be used in conjunction with the Neural Stem Cell Marker Characterization Kit (SCR019) and differentiation assays that demonstrate multipotentiality of the starting cell population. Please look for these differentiation assays from in the near future.

For Research Use Only; not for use in diagnostic procedure.

Kit Components

1x10^6 viable Mouse Cortical Neural Stem Cells (2004115)

Derived from C57/BL6 embryonic day 15-18 mice, cryopreserved. Store in liquid nitrogen.

Characterization of Cells

Each lot of primary mouse cortical neural stem cells has been validated for high level of expression of Nestin and Sox 2 and for their self-renewal and multi-lineage differentiation capacities (please refer to insert figures). Cells tested negative for mycoplasma.

Materials Required (Not Supplied)

- Neural Stem Cell Expansion Medium (SCM003)
- Basic fibroblast growth factor (bFGF, FGF-2; Specific Activity > $2x10^6$ units/mg; GF003)
- Epidermal Growth Factor (EGF; Specific Activity > $1x10^7$ units /mg; GF001)
- Heparin (H L027)
- Poly-L-ornithine (P3655)
- Laminin (L 2020)
- ESGRO Complete Accutase® (SF006)
- Tissue culture-ware
- Phosphate-Buffered Saline (1X PBS) (BSS-1005-B)
- Fixative (for example, 4% Paraformaldehyde in 1X PBS)
- Blocking Solution (5% normal donkey serum, 0.3% Triton™ X-100 in 1X PBS)
- Primary and secondary antibodies
- 4'-6-Diamidino-2-phenylindole (DAPI)/PBS solution
- Anti-fading mounting solution (DABCO/PVA)
- Hemacytometer
- Microscope

Storage

Store in liquid nitrogen. Do not expose to elevated temperatures. We recommend that the cells be used within ten passages after thaw.

Preparation of Coated Plates

We recommend coating tissue culture plastic- or glassware that are used to culture mouse cortical 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 or –80 °C.
2. Dilute poly-L-ornithine with water from the stock concentration (10 mg/mL) to yield:
   - 10 µg/mL for polystyrene plates
   - 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-ware 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 mouse cortical 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.
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 Expansion Medium (pre-warmed to 37 °C) to the 15 mL conical tube.

   IMPORTANT: Do not add the whole volume of media at once to the cells. This may result in decreased cell viability due to osmotic shock**.**
5. Gently mix the cell suspension by slow pipetting up and down twice. Be careful to not introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
6. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
7. Decant as much of the supernatant as possible. Steps 4-6 are necessary to remove any residual cryopreservative (DMSO).
8. Resuspend the cells in a total volume of 10 mL of Neural Stem Cell Expansion Medium (pre-warmed to 37 °C) containing freshly added 20 ng/mL FGF-2, 20 ng/mL EGF, and 2 µg/mL heparin.

   NOTE: FGF-2, EGF and heparin should always be added fresh to the Neural Stem Cell Expansion Medium.
9. Plate the cell mixture onto a poly-L-ornithine and laminin-coated 10 cm tissue culture plate.
10. Incubate the cells at 37 °C in a 5% CO_2 humidified incubator.
11. The next day, exchange the medium with fresh Neural Stem Cell Expansion Medium (pre-warmed to 37 °C) containing 20 ng/mL FGF-2, 20 ng/mL EGF and 2 µg/mL heparin. Exchange with fresh medium containing FGF-2, EGF, and heparin every other day thereafter.
12. 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 mouse cortical neural stem cells.
2. Apply 3-5 mL of Accutase® 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 Expansion 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 x *g* for 2-3 minutes to pellet the cells.
7. Discard the supernatant.
8. Apply 2 mL of Neural Stem Cell Expansion Medium containing 20 ng/mL FGF-2, 20 ng/mL EGF, and 2 µg/mL heparin to the conical tube and resuspend the cells thoroughly.
9. Count the number of cells using a hemocytometer.
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 Expansion Medium containing 20 ng/mL FGF-2, 20 ng/mL EGF, and 2 µg/mL heparin. We typically plated the cells at ~2 million cells on poly-L-ornithine and laminin coated 10 cm plates or T75 flasks.

Characterization of Mouse Cortical Neural Stem Cells (SCR029)

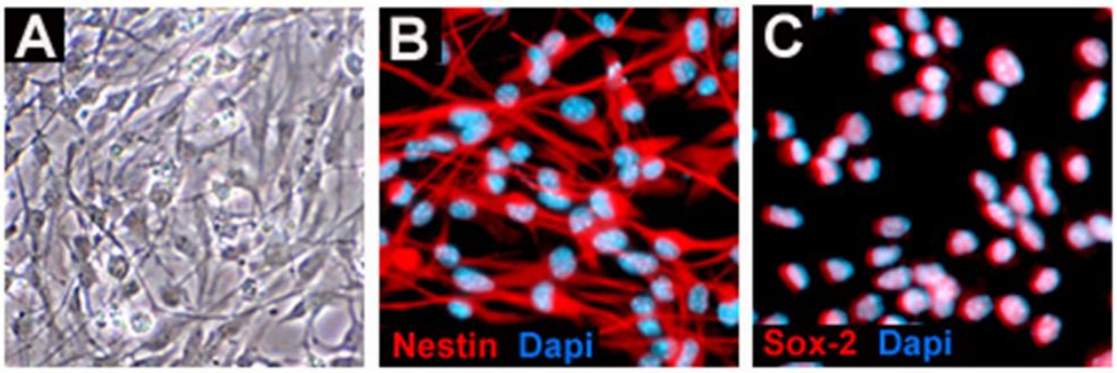

Figure 1. Mouse Cortical NSC (SCR029) are grown as monolayers (**A**) and express NSC markers, Nestin (**B**) and Sox-2 (**C**). Nuclei of the cells were visualized with DAPI (blue). The Sox-2 transcription factor is co-localized with the DAPI (blue) staining in the nucleus (**C**). Mouse cortical NSC are derived from embryonic day 15-18 C57/BL mice. Isolated cortices were diced into small fragments, enzymatically dissociated, and propagated on poly-L-ornithine and laminin-coated tissue culture plates in Neural Stem Cell Expansion Medium containing 20 ng/mL FGF-2, 20 ng/mL EGF, and 2 µg/mL heparin.

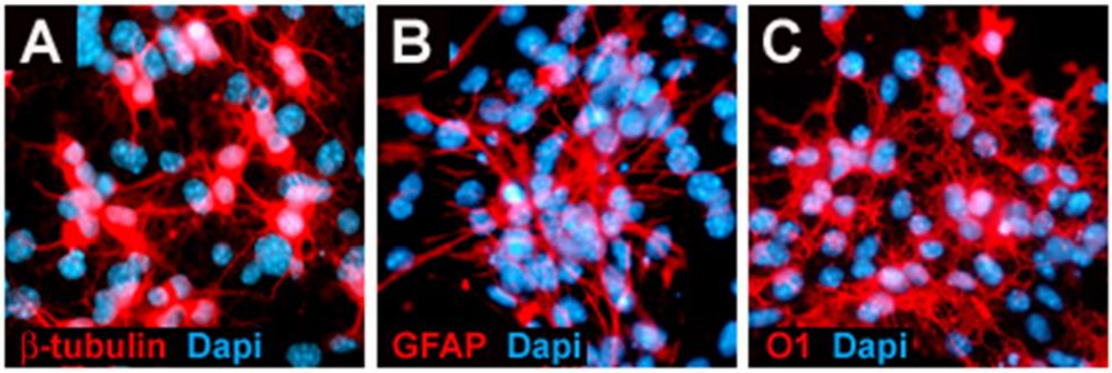

Figure 2. Mouse cortical NSC are multipotent. NSC cultured in the presence of retinoic acid and forskolin differentiate into neurons (β-tubulin; **A**), astrocytes (GFAP; **B**) and oligodendrocytes (O1; **C**). Nuclei of the cells were visualized with DAPI (blue).

Related Products

The following stem cell products are available as separate items:

- Cryopreserved Mouse Spinal Cord Neural Stem Cells (SCR031)
- Adult Rat Hippocampal Neural Stem Cell Kit (SCR021)
- Cryopreserved Adult Rat Hippocampal Neural Stem Cells (SCR022)
- Neural Stem Cell Expansion Medium (SCM003)
- Neural Stem Cell Marker Characterization Kit (SCR019)
- Neuron-Glial Marker Sampler Kit (NS130)
- Embryonic Stem Cell Derived Neuron Integration and Characterization Kit (NS140)
- Dopaminergic Neuron Integration and Characterization Kit (NS145)
- Rat Hippocampal Astrocyte Kit (SCR007)
- Cryopreserved Rat Hippocampal Astrocytes (SCR008)
- Rat Hippocampal Neuron Kit (SCR009)
- Cryopreserved Rat Hippocampal Neurons (SCR010)
- Mouse-anti Nestin, 100 µg (MAB353)
- Rabbit-anti Sox-2, 100 µg (AB5603)
- Mouse-anti Map2ab, 200 µg (MAB3418)
- Mouse-anti Oligodendrocyte marker O1, 50 µg (MAB344)
- Mouse-IgM, purified 1 mg (PP50)
- Mouse-IgG, purified 10 mg (PP54)
- Rabbit-IgG, purified 25 mg (PP64)

Notice

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