

MILLIPORE

MilliTrace™
Constitutive GFP Reporter
Adult Rat Hippocampal
Neural Stem Cell Kit

Cat. No. SCR080

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

See Use Restrictions contained herein

USA & Canada Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209
Europe +44 (0) 23 8026 2233 Australia +61 3 9839 2000
www.millipore.com

Introduction

Millipore's MilliTrace™ Constitutive GFP Reporter Adult Rat Hippocampal Neural Stem Cell Kit (Catalog No. SCR080) provides ready-to use primary rat neural stem cells that are constitutively labeled with the humanized mulleri green fluorescent protein (hmGFP) along with expansion medium to help maintain expression of the transgene. hmGFP expression is driven off a constitutive promoter, chicken actin. These stable GFP transfected cells have been FACS sorted to over 95-100% purity.

The constitutive GFP Reporter Adult Rat Hippocampal NSCs display the immunochemical staining properties of NSC; they are Nestin⁺Sox2⁺TuJ1⁺GFAP⁻. These cells are multipotent. Under defined neuronal differentiation conditions (Rodent Neuron Differentiation Kit, Millipore Cat. No. SCR035), the majority of cells are differentiated to βIII-tubulin positive neurons (GFP⁺βIII-tubulin⁺GFAP⁻). Under defined astrocytic differentiation conditions (Astrocyte Differentiation Medium, Millipore Cat. No. SCM010), majority of cells differentiate to GFAP-positive astrocytes (GFP⁺GFAP⁺βIII-tubulin⁻). These cells display normal karyotype as assessed by chromosomal spread (>90% cells have normal 42 chromosomes) and are confirmed to be mycoplasma-free.

We recommend that the MilliTrace Constitutive GFP Reporter Adult Rat Hippocampal Neural Stem Cell Kit (Catalog No. SCR080) be used in conjunction with the Neural Stem Cell Marker Characterization Kit (Catalog No. SCR019) and differentiation assays that demonstrate multipotentiality of the starting cell population, Rodent Neuron Differentiation Kit (Catalog No. SCR035) and Rodent Astrocyte Differentiation Medium (Catalog No. SCM010).

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Kit Components

1. 1 x 10⁶ viable MilliTrace Constitutive GFP Reporter Adult Rat Hippocampal Neural Stem Cells: (Catalog No. SCC080) derived from adult female Fisher rat, cryopreserved. Store in liquid nitrogen.
2. MilliTrace Rat Neural Stem Cell Expansion Medium: (Catalog No. SCM040) contains 500 mL Neural Stem Cell Basal Medium (Part No. SCM003), 5 mg/mL Puromycin Solution (Part No. CS201361) and 10 µg FGF-2, lyophilized (Part No. GF003-10UG). Store at -20°C.

Characterization of Cells

Primary constitutive GFP reporter rat neural stem cells have been validated for high level of constitutive expression of GFP, high level of expression of Nestin and Sox 2, and for their self-renewal and multi-lineage differentiation capacities (please refer to insert figures for representative data). Cells display normal karyotype as assessed by chromosome spread and tested negative for mycoplasma.

Materials Required But Not Provided

1. Poly-L-ornithine (Sigma Catalog No. P3655)
2. Laminin (Catalog No. CC095)
3. Accutase™ Cell Dissociation Solution (Catalog No. SCR005)
4. Tissue culture-ware
5. Glass coverslips
6. Phosphate-Buffered Saline (1X PBS) (Catalog No. BSS-1005-B)
7. EmbryoMax ES Cell Qualified Ultra Pure Water, sterile H₂O, 500 mL (Catalog No. TMS-006-B)
8. Fixative (e.g. 4% Paraformaldehyde in 1X PBS)
9. Blocking Solution (5% normal donkey serum, 0.3% Triton X-100 in 1X PBS)
10. Neural Stem Cell Characterization Kit (Catalog No. SCR019)
11. Rodent Neuron Differentiation Kit (Catalog No. SCR035)
12. Rodent Astrocyte Differentiation Kit (Catalog No. SCM010)

13. Fluorescent-labeled secondary antibodies. Donkey anti-mouse IgG, Cy3 conjugated (Catalog No. AP192C) and donkey anti-rabbit IgG, Cy3 conjugated (Catalog No. AP182C).
14. 4'-6-Diamidino-2-phenylindole (DAPI) / PBS solution
15. Nunc Lab-Tek II 8 well chamber slides (Fisher Catalog No. 12-565-8)
16. Anti-fading mounting solution (DABCO/PVA)
17. Hemacytometer
18. Microscope

Storage

MilliTrace Constitutive GFP Reporter Adult Rat Hippocampal Neural Stem Cells (Part No. SCC080) should be stored in Liquid Nitrogen. We recommend that the cells be used within ten passages.

Neural Stem Cell Basal Medium (Part No. SCM003) should be stored at -20°C until expiration date on the label. Upon thawing the basal medium should be stored at 2-8°C and given a 1-month expiration dating.

5 mg/mL Puromycin Solution, 100 µL (Part No. CS201361) should be stored in working aliquots at -20°C for up to 2 years.

FGF-2 (10 µg, 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.

Preparation of Coated Plates

We recommend coating tissue culture plastic- or glassware that are used to culture rat 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-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

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 constitutive GFP reporter adult rat hippocampal 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 Neural Stem Cell Basal Medium (Catalog No. SCM003) (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.**

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 4-6 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in a total volume of 10 mL Neural Stem Cell Basal Medium (Catalog No. SCM003) (pre-warmed to 37°C) 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.*
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% CO₂ humidified incubator.
12. The next day, exchange the medium with fresh Neural Stem Cell Basal Medium (pre-warmed to 37°C) containing 20 ng/mL FGF-2 and 1 µg/mL puromycin. Exchange with fresh medium containing FGF-2 and puromycin every other day thereafter.
13. When the cells are approximately 80% confluent, they can be dissociated with Accutase cell dissociation 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 constitutive GFP reporter adult rat hippocampal 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 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 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.
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 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.

Results

Characterization of Constitutive GFP Reporter Adult Rat Hippocampal Neural Stem Cells (Catalog No. SCC080)

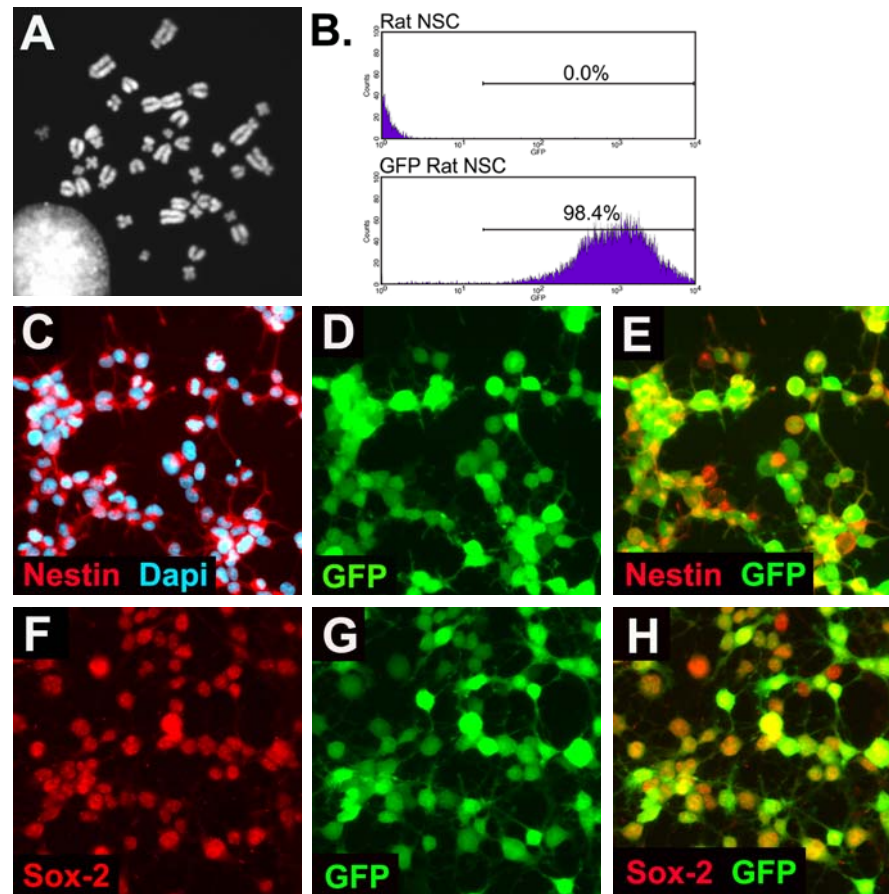


Figure 1. Constitutive GFP Reporter Adult Rat Hippocampal Neural Stem Cells (Catalog No. SCC080) have a normal karyotype (A) and constitutively express GFP (B, D, G) along with NSC markers, Nestin (C, E) and Sox-2 (F, H). Nuclei of the cells were visualized with DAPI (blue). The Sox-2 transcription factor is co-localized with the GFP staining in the nucleus. Majority of cells are GFP-positive (B, D, G).

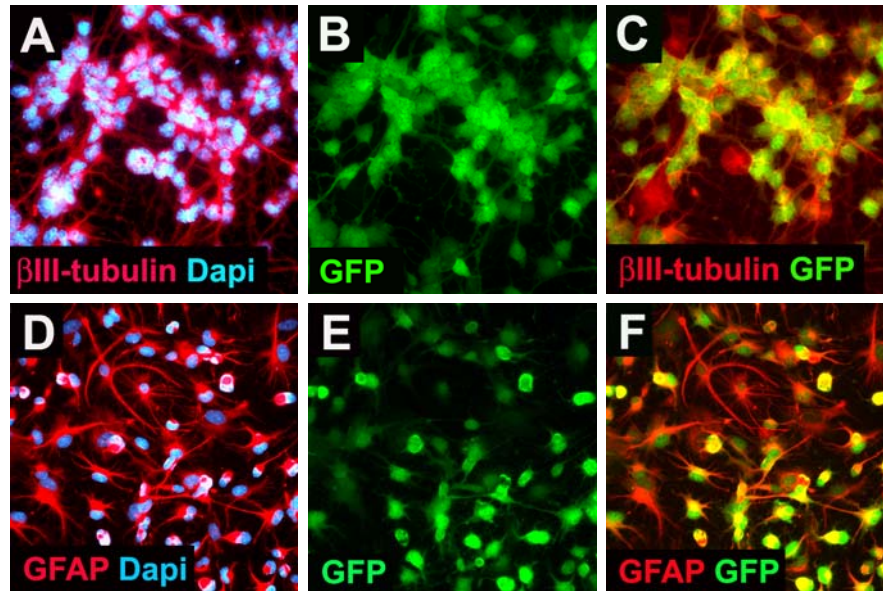


Figure 2. Constitutive GFP Reporter Adult Rat Hippocampal Neural Stem Cells are multipotent. Using Rodent Neuron Differentiation Kit (Catalog No. SCR035), NSC can differentiate into neurons (β III-tubulin, **A, C**, red). Using Rodent Astrocyte Differentiation Medium (Catalog No. SCM010) NSC can differentiate into astrocytes (GFAP, **D, F**, red). Majority of cells are GFP-positive (**B, E**).

*For color images, please go to www.millipore.com

References

1. Gage, F. H. (2000). Mammalian neural stem cells. *Science* **287**: 1433-1438.
2. Palmer, T. D., Takahashi, J., and Gage, F. H. (1997). The adult rat hippocampus contains primordial neural stem cells. *Mol. Cell. Neurosci.* **8(6)**: 389-404.
3. Palmer, T. D., Markakis, E. A., Wilhoite, A. R., and Safar, F., and Gage, F. H. Fibroblast growth factor-2 activates a latent neurogenic program in

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4. Lendahl, U., Zimmerman, L. B. & McKay R. D. (1990). CNS stem cells express a new class of intermediate filament protein. *Cell* **60**: 585-595.
5. Graham, V. Khudyakov, J., Ellis, P., and Pevny, L. (2003). Sox2 functions to maintain neural progenitor identity. *Neuron* **39 (5)**: 749-65.

Related Products

The following products are available from Millipore as separate items:

1. MilliTrace Rat Neural Stem Cell Expansion Medium: (Catalog No. SCM040)
2. MilliTrace Rodent Neural Stem Cell Basal Medium: (Catalog No. SCM060)
3. Neural Stem Cell Basal Medium: (Catalog No. SCM003)
4. Basic Fibroblast Growth Factor (FGF-2): (Catalog No. GF003)
5. Laminin, mouse: (Catalog No. CC095)
6. Rodent Neuron Differentiation Kit: (Catalog No. SCR035)
7. Rodent Astrocyte Differentiation Kit: (Catalog No. SCM010)
8. Neural Stem Cell Marker Characterization Kit: (Catalog No. SCR019)
9. Neuron-Glial Marker Sampler Kit: (Catalog No. NS130)
10. Embryonic-Stem Cell Derived Neuron Integration and Characterization Kit: (Catalog No. NS140)
11. Dopaminergic Neuron Integration and Characterization Kit: (Catalog No. NS145)
12. Mouse anti-Nestin, 100 µg: (Catalog No. MAB353)
13. Rabbit anti-Sox-2, 100 µg: (Catalog No. AB5603)
14. Mouse anti-βIII tubulin, 100 µL: (Catalog No. MAB1637)
15. Rabbit anti-GFAP, 50 µL: (Catalog No. AB5804)

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