

# MILLIPORE

## **Neural Stem Cell Characterization Kit**

**Catalog No. SCR019**

**FOR RESEARCH USE ONLY**  
**Not for use in diagnostic procedures**

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## Introduction

Neural stem cells are present in both the developing and adult nervous system of all mammals, including humans (1). They possess the remarkable capacity to self-renew and to differentiate along specific pathways to generate the vast array of neuronal and glial cell types of the central nervous system (CNS). 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 are correlated with the stem and/or progenitor state along with the absence of a more differentiated phenotype as assessed through marker analysis. To aid researchers in the accurate identification of neural stem cells, Millipore offers the Neural Stem Cell Characterization Kit (Catalog Number SCR019).

The Neural Stem Cell Characterization Kit contains two molecular markers, Nestin (2) and Sox 2 (3), that are frequently used to identify neural stem/progenitor cells along with more differentiated lineage markers including Map2ab for neurons, GFAP for astrocytes, and O1 for oligodendrocytes (see table below). Mouse and rabbit immunoglobulins for the assessment of background staining are also included. All of the antibodies provided in the kit have been tested and optimized for use in immunocytochemistry on adult rat neural stem cells.

Identification	Sox2	Nestin	MAP-2	GFAP	O1
Pluripotent stem cell	+	-	-	-	-
Neural stem cell	+	+	-	-	-
Neuronal lineage	-	-	+	-	-
Astrocyte lineage	-	-	-	+	-
Oligodendrocyte lineage	-	-	-	-	+

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## Kit Components

1. Mouse anti-Nestin: (Catalog No. 2003602) One vial containing 50 µg monoclonal antibody. Store at 2° to 8°C.
2. Rabbit anti-Sox 2: (Catalog No. 2003600) One vial containing 20 µg affinity purified polyclonal antibody. Store at 2° to 8°C.
3. Mouse anti-MAP-2: (Catalog No. MAB3418-50UG) One vial containing 50 µg monoclonal antibody. Store at 2° to 8°C.
4. Rabbit anti-GFAP: (Catalog No. AB5804) One vial containing 50 µL of polyclonal rabbit serum. Store at -20 °C.
5. Mouse anti-Oligodendrocyte marker O1: (Catalog No. 2003601) One vial containing 20 µg IgM monoclonal antibody. Store at 2-8 °C.
6. Mouse IgM: (Catalog No. 2003599) One vial containing 50 µg purified mouse IgM control antibody. Store at 2° to 8°C.
7. Mouse IgG: (Catalog No. PP54-100UG) One vial containing 100 µg purified mouse IgG control antibody. Store at -20 °C.
8. Rabbit IgG: (Catalog No. PP64-100UG) One vial containing 100 µg purified rabbit IgG antibody. Store at -20 °C.

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## Materials Not Supplied

1. Adult Rat Neural Stem Cell Expansion Medium (Catalog No. SCM009)
  2. Mouse Neural Stem Cell Expansion Medium (Catalog No. SCM008)
  3. Chamber slides
  4. Glass coverslips
  5. Phosphate-Buffered Saline (1X PBS) (Catalog No. BSS-1005-B)
  6. Fixative (e.g. 4% Paraformaldehyde in 1X PBS)
  7. Blocking Solution (5% normal donkey serum, 0.3% Triton X-100 in 1X PBS)
  8. Non-Permeable Blocking Solution (5% normal donkey serum in 1X PBS)
  9. Fluorescent-labeled secondary antibodies. Donkey anti-mouse IgG, Cy3 conjugated (Cat. No. AP192C) and donkey anti-rabbit IgG, Cy3 conjugated (Cat. No. AP182C) are recommended
  10. 4'-6-Diamidino-2-phenylindole (DAPI) / PBS solution
  11. Anti-fading mounting solution (DABCO/PVA)
  12. Hemacytometer
  13. Microscope
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## Storage

When stored at the recommended storage conditions (refer to Kit Components), components are stable up to the expiration date. Do not expose to elevated temperatures. Discard any remaining reagents after the expiration date.

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## Staining Protocol (for 8-well chamber slides)

1. Culture the neural stem cells in Adult Rat Neural Stem Cell Expansion Medium until the cells are 60-70% confluent. For 8 well chamber slides, this corresponds to 100,000 cells per well in proliferating culture medium.
2. The next day, carefully aspirate the media and fix the cells with a fixative (i.e. 4% paraformaldehyde in 1X PBS). Be careful to not aspirate the cells.
3. Incubate in 4% paraformaldehyde for 30-40 minutes at room temperature.
4. Carefully aspirate the fixative and rinse three times (5-10 minutes each) with 1X PBS.
5. Apply a blocking solution for at least 2 hours at room temperature or overnight at 4°C. **IMPORTANT: Do not shake the cells.** For optimal results, use the Blocking Solution (5% Normal donkey serum, 0.3% Triton X-100 in 1X PBS) with antibodies directed against Nestin, Sox 2, Map2ab and GFAP. Use the Non-Permeable Blocking Solution (5% Normal donkey serum in 1X PBS) with the antibody directed against the Oligodendrocyte marker, O1.
6. Dilute the primary antibodies included in this kit to working concentrations in the appropriate blocking solutions. For optimal results, the following antibody dilutions are recommended for immunocytochemistry (see images):

Mouse anti-Nestin: 1/200 dilution based on 1 mg/mL, final 5 ng/μL

Rabbit anti-Sox 2: 1/1000 dilution based on 1 mg/mL, final 1 ng/μL

Mouse anti-MAP-2: 1/200 dilution based on 1 mg/mL, final 5 ng/μL

Rabbit anti-GFAP: 1/250 dilution of rabbit serum

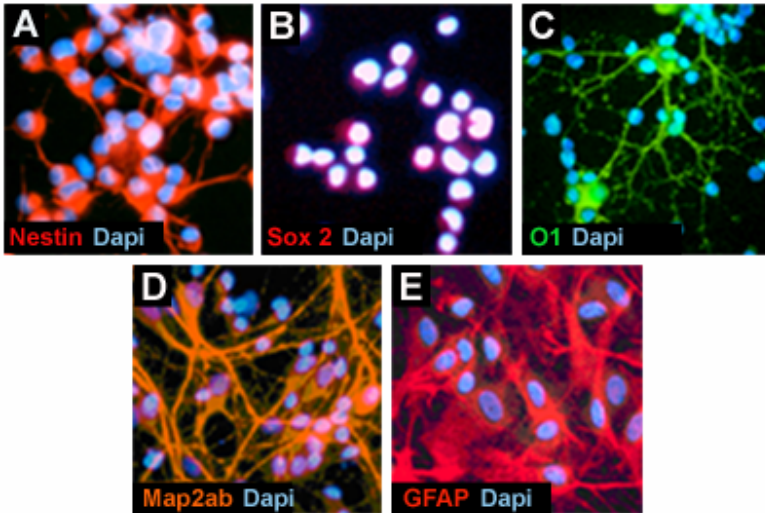
Mouse anti- O1: 1/500 dilution based on 1 mg/mL, final 2 ng/μL

7. In a separate control well, depending upon the specific antibody used, add equivalent concentrations of mouse IgG (1 mg/mL), rabbit IgG (1 mg/mL) or mouse IgM (2 mg/mL) to 0.5 mL of the appropriate blocking solution. For example, to obtain a 1/500 dilution of mouse anti-Oligodendrocyte, O1 (1 mg/mL) IgM antibody, 1  $\mu$ L of the antibody is added to 0.5 mL volume of the Non-Permeable Blocking Solution. In an adjacent control well, add 1  $\mu$ L mouse IgM (1 mg/mL) control antibody to 0.5 mL of the Non-Permeable Blocking Solution.
8. Incubate the cells in primary antibodies overnight at 4°C. **IMPORTANT: Do not shake.**
9. The next day, wash the cells twice with 1X PBS (5-10 minutes each wash) and twice with the appropriate blocking solution.
10. At the completion of the last wash, leave the cells in blocking solution for at least 30 minutes.
11. Dilute secondary antibodies in the appropriate blocking solution just before use. The following secondary antibodies can be used: donkey anti-mouse IgG Cy3 conjugated (Cat. No. AP192C), donkey anti-mouse IgG FITC conjugated (Cat. No. AP192F), donkey anti-rabbit IgG Cy3 conjugated (Cat. No. AP182C), donkey anti-rabbit IgG FITC conjugated (Cat. No. AP182F), and donkey anti-mouse IgM Cy3 conjugated (Jackson Laboratories) antibodies at a 1:250 or 1:500 dilution.
12. Overlay the cells with the appropriate donkey anti-mouse and anti-rabbit secondary antibodies that are conjugated to fluorescent molecules for 2 hours at room temperature.
13. Wash 3-5 times (5-10 minutes each) with 1X PBS.
14. Counterstain the cell nuclei with DAPI / 1X PBS solution.
15. Mount a glass coverslip over the chamber slides using antifading mounting solution (e.g. DABCO/PVA).
16. Visualize the cell staining with a fluorescent microscope.  
**Note:** *Be sure to use the correct filter to visualize fluorescent-labeled cells.*

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## Results

### Immunofluorescent Images of Antibody Components in Neural Stem Cell Characterization Kit (SCR019)\*



Figures A-E. Cultured adult rat hippocampus-derived neural stem cells stained for (A) Nestin (red) and (B) Sox-2 (red). The Sox-2 transcription factor is colocalized with the DAPI (blue) staining in the nucleus. C. Mouse anti-Oligodendrocyte O1 (green) staining of adult rat hippocampus-derived neural stem cells that have been exposed to differentiation conditions for four days. D. Localization of MAP-2 (orange) in primary rat hippocampus-derived embryonic neurons (Catalog No. SCR010) that have been thawed and cultured for ten days. E. Primary rat hippocampus-derived embryonic astrocytes (Catalog No. SCR008) stained for GFAP (red). Nuclei of the cells were visualized with DAPI (blue).

Please note that developmental stem cell marker expression is not necessarily mutually exclusive during transitional states and some markers may colocalize for brief periods. For more information about the use of stem cell and differentiated tissue markers, detailed information on additional applications for our markers and journal references can be viewed online using the catalog numbers listed in the Related Products section of this insert.

\*For color images, please go to [www.millipore.com](http://www.millipore.com)

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## References

1. Gage, F. H. (2000). Mammalian neural stem cells. *Science* **287**: 1433-1438.
2. Lendahl, U., Zimmerman, L. B. & McKay R. D. (1990). CNS stem cells express a new class of intermediate filament protein. *Cell* **60**: 585-595.
3. Graham, V. Khudyakov, J., Ellis, P., and Pevny, L. (2003). Sox2 functions to maintain neural progenitor identity. *Neuron* **39 (5)**: 749-65.

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## Related Products

The following products are available from Millipore as separate items:

1. Adult Rat Hippocampal Neural Stem Cells: (Catalog No. SCR022)
2. Mouse Cortical Neural Stem Cells: (Catalog No. SCR029)
3. Mouse Spinal Cord Neural Stem Cells: (Catalog No. SCR031)
4. Cryopreserved Rat Hippocampal Astrocytes: (Catalog No. SCR008)
5. Cryopreserved Rat Hippocampal Neurons: (Catalog No. SCR010)
6. Neuron-Glial Marker Sampler Kit: (Catalog No. NS130)
7. Embryonic Stem Cell Derived Neuron Integration and Characterization Kit: (Catalog No. NS140)
8. Mouse-anti Nestin, 100 µg: (Catalog No. MAB353)
9. Rabbit-anti Sox-2, 100 µg: (Catalog No. AB5603)
10. Mouse-anti Map2ab, 200 µg: (Catalog No. MAB3418)
11. Mouse-anti Oligodendrocyte marker O1, 50 µg: (Catalog No. MAB344)
12. Mouse-IgM, purified 1mg: (Catalog No. PP50)
13. Mouse-IgG, purified 10 mg: (Catalog No. PP54)
14. Rabbit-IgG, purified 25 mg: (Catalog No. PP64)

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## Warranty

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