Human Neural Stem Cell Characterization Kit

Catalog No. SCR060

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Not for use in diagnostic procedures.

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Introduction

Neural stem cells are present in both the developing and adult nervous systems 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 human neural stem cells, Millipore presents the Human Neural Stem Cell Characterization Kit (Catalog Number SCR060).

Millipore's Human Neural Stem Cell Characterization Kit (Catalog Number SCR060) contains three molecular markers, Nestin (2), Sox 2 (3) and Musashi (4) that are frequently used to identify neural stem/progenitor cells along with more differentiated lineage markers including βIII-tubulin for neurons, GFAP for astrocytes and O1 for oligodendrocytes. 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 human neural stem cells. We recommend that Millipore's Human Neural Stem Cell Characterization Kit be used in conjunction with differentiation assays that demonstrate multipotentiality of the starting cell population.

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Kit Components

1. Mouse anti-Nestin, Human specific: (Part No. MAB5326-50UG) One vial containing 50 µg monoclonal antibody. Store at 2° to 8°C.
2. Rabbit anti-Sox 2: (Part No. 2003600) One vial containing 20 µg affinity purified polyclonal antibody. Store at 2° to 8°C.
3. Rabbit anti-Musashi: (Part No. AB5977-50UG) One vial containing 50 µg polyclonal antibody. Store at -20°C or -70°C.
4. Mouse anti-βIII Tubulin: (Part No. MAB1637-50UL) One vial containing 50 µL monoclonal antibody. Store at -20°C.
5. Rabbit anti-GFAP: (Part No. AB5804) One vial containing 50 µL of polyclonal rabbit serum. Store at –20 °C.
6. Mouse anti-Oligodendrocyte marker O1: (Part No. 2003601) One vial containing 20 µg IgM monoclonal antibody. Store at 2-8°C.
7. Mouse IgM: (Part No. 2003599) One vial containing 50 µg purified mouse IgM control antibody. Store at 2° to 8°C.
8. Mouse IgG: (Part No. PP54-100UG) One vial containing 100 µg purified mouse IgG control antibody. Store at -20 °C.
9. Rabbit IgG: (Part No. PP64-100UG) One vial containing 100 µg purified rabbit IgG antibody. Store at -20 °C.

Materials Not Supplied

1. Human neural stem cells
2. Culture reagents
3. Chamber slides
4. Basic fibroblast growth factor (bFGF; FGF-2; Specific Activity $\geq$ 2 X 10^6 Units/mg. Millipore Cat. No. GF003)
5. Epidermal growth factor (EGF; Specific Activity $\geq$ 1 x 10^7 Units/mg; Millipore Cat. No. GF001)
6. Laminin (Sigma Cat. No. L-2020)
7. Accutase™ (Millipore Cat. No. SCR005)
8. Tissue culture-ware
9. Glass coverslips
10. Phosphate-Buffered Saline (1X PBS) (Millipore Cat. No. BSS-1005-B)
11. Fixative (e.g. 4% Paraformaldehyde in 1X PBS)
12. Blocking Solution (5% normal donkey serum, 0.3% Triton X-100 in 1X PBS)
13. Non-Permeable Blocking Solution (5% normal donkey serum in 1X PBS)
14. Fluorescent-labeled secondary antibodies. Donkey anti-mouse IgG, Cy3 conjugated (Millipore Cat. No. AP192C), donkey anti-rabbit IgG, Cy3 conjugated (Millipore Cat. No. AP182C) and donkey anti-mouse IgM, Cy3 conjugated (Jackson Laboratories Cat. No. 715-165-140) are recommended
15. 4'-6-Diamidino-2-phenylindole (DAPI) / PBS solution
16. Anti-fading mounting solution (DABCO/PVA)
17. Hemacytometer
18. Microscope

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.

Staining Protocol (for 8-well chamber slides)

1. Culture the human neural stem cells in the appropriate expansion medium with growth factors 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 15-20 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, Musashi, βIII-tubulin 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: 1/500 dilution of 1 mg/mL, final 2 µg/mL

   Rabbit anti-Sox 2: 1/1000 dilution of 1 mg/mL, final 1 µg/mL

   Rabbit anti-Musashi: 1/500 dilution of 1 mg/mL, final 2 µg/mL

   Mouse anti-βIII tubulin: 1/1000 dilution of monoclonal antibody

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

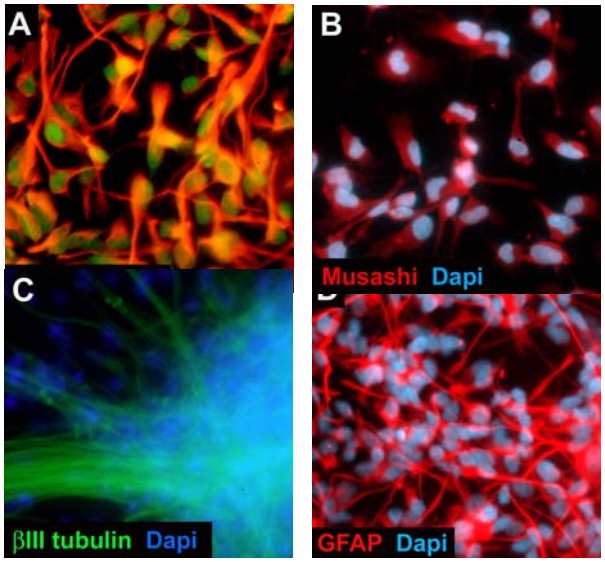
   Mouse anti- O1: 1/500 dilution of 1 mg/mL, final 2 µg/mL
7. In a separate control well, depending upon the specific antibody used, add equivalent concentrations of mouse IgG (1 mg/mL), rabbit IgG (1 mg/mL) or mouse IgM (2 mg/mL) to 0.5 mL of the appropriate blocking solution. For example, to obtain a 1/500 dilution of mouse anti-Oligodendrocyte, O1 (1 mg/mL) IgM antibody, 1 µL of the antibody is added to 0.5 mL volume of the Non-Permeable Blocking Solution. In an adjacent control well, add 1 µL 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 (Millipore Cat. No. AP192C), donkey anti-mouse IgG FITC conjugated (Millipore Cat. No. AP192F), donkey anti-rabbit IgG Cy3 conjugated (Millipore Cat. No. AP182C), donkey anti-rabbit IgG FITC conjugated (Millipore Cat. No. AP182F), and donkey anti-mouse IgM Cy3 conjugated (Jackson Laboratories) antibodies at a 1:250 to 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.*

Immunofluorescent Images of Antibody Components in Human Neural Stem Cell Characterization Kit (SCR060)



ReNcell CX immortalized human cortical neural progenitor cells (MILLIPORE Cat. No. SCC007) stained for NSC markers, Nestin (**A**, red), Sox-2 (**A**, green) and Musashi (**B**, red). The Sox-2 transcription factor is localized in the cell nucleus. Human neural progenitor cells were differentiated into neurons (III-tubulin; **C,** green) and glial cells (GFAP; **D,** red). Nuclei of the cells were visualized with DAPI (blue).

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

Identification	Sox2	Nestin	Musashi	III-tubulin	GFAP	O1
Pluripotent Stem Cell	+	-	-	-	-	-
Neural Stem Cell	+	+	+	-	-	-
Neuronal Lineage	-	-	-	+	-	-
Astrocyte Lineage	-	-	-	-	+	-
Oligodendrocyte Lineage	-	-	-	-	-	+

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, visit the Millipore website and download our Stem Cell Biology, Cell Culture, and Nervous System Development & Differentiation technical brochures. 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.

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.
4. Sakakibara, S., Imai, T., Aruga, J., Nakajima, K., Yasutomi, D., Nagata, T., Kurihara, Y., Uesugi, S., Miyata,, T., Ogawa, M., Mikoshiba, K., and Okano, H. (1996). Mouse-Musashi-1, a neural RNA-binding protein highly enriched in the mammalian CNS stem cell. *Dev. Biol.* **176**: 230-242.

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