MILLIPORE

Human Embryonic Stem Cell Neurogenesis Characterization Kit

Catalog No. SCR065

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Not for use in diagnostic procedures

USA & Canada
Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000 • Germany +49-6192-207300 • ISO Registered worldwide
www.Millipore.com

Introduction

Embryonic stem cells (ES cells) are derived from pluripotent cells of the early embryo and have the potential to differentiate into a variety of cell types including neurons. The derivation of neural progenitor cells from human ES cells is of great value both as a critical tool to facilitate the study of early human neurogenesis and as a potential renewable source of donor cells for neural transplantation therapies. Presently, human ES 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 ES cells and their differentiated neural lineages, Millipore presents the Human Embryonic Stem Cells Neurogenesis Characterization Kit (Catalog No. SCR065).

Millipore's Human Embryonic Stem Cell Neurogenesis Characterization Kit contains a complete panel of validated antibodies that allows researchers to identify and quantify the extent of differentiation to specific neuronal subtypes from a starting culture of human embryonic stem cells. Pluripotent markers, Oct-4, SSEA-4 and Sox-2 are provided in the kit to aid in the characterization of the starting human embryonic stem cell culture. To characterize the transition of human ES cells from pluripotent to multipotent state with the potentiality restricted to cells of the neural lineage, Nestin and Sox-2 is provided. βIII-tubulin antibody is provided to mark all neuronal cells while GAD67, ChAT and TH antibodies are provided to specifically identify GABAergic, cholinergic, and dopaminergic neurons, respectively.

All of the antibodies provided in the kit have been tested and optimized for use in immunocytochemistry on human embryonic stem cells and human-derived neural stem cells. We recommend that Millipore's Human Embryonic Stem Cell Neurogenesis Characterization Kit be used in conjunction with differentiation assays that demonstrate multipotentiality of the starting cell population.

Identification	Oct 4	SSEA-4	Sox-2	Nestin	βIII tubulin	GAD67	ChAT	TH
Pluripotent Stem Cell	+	+	+	-	-	-	-	-
Neural Stem Cell	-	-	+	+	-	-	-	-
Pan-Neuronal Lineage	-	-	-	-	+	-	-	-
GABAergic Neurons	-	-	-	-	+	+	-	-
Cholinergic Neruons	-	-	-	-	+	-	+	-
Dopaminergic Neurons	-	-	-	-	+	-	-	+

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.

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Kit Components

1. Mouse anti-Oct 4: (Catalog No. MAB4401-50UG) One vial containing 50 µg monoclonal antibody. Store at 2° to 8°C.
2. Mouse anti-SSEA-4: (Catalog No. MAB4304-50UG) One vial containing 50 µg monoclonal antibody. Store at 2° to 8°C.
3. Mouse anti-Nestin, Human specific: (Catalog No. MAB5326-10UG) One vial containing 10 µg monoclonal antibody. Store at 2° to 8°C.
4. Rabbit anti-Sox 2: (Catalog No. AB5603-10UG) One vial containing 10 µg affinity purified polyclonal antibody. Store at 2° to 8°C.
5. Mouse anti-βIII Tubulin: (Catalog No. MAB1637-10UL) One vial containing 10 µL monoclonal antibody. Store at -20°C.
6. Rabbit anti-GAD67: (Catalog No. AB9706-10UG) One vial containing 10 µg polyclonal antibody. Store at 2° to 8°C.
7. Goat anti-ChAT: (Catalog No. AB144P-50UL) One vial containing 50 µL of polyclonal antibody. Store at –20°C.
8. Rabbit anti-Tyrosine Hydroxylase (TH): (Catalog No. AB152-20UL) One vial containing 20 µL rabbit polyclonal antibody. Store at at –20°C.

Materials Required But Not Provided

1. Human embryonic stem cells
2. Culture medium and reagents
3. Chamber slides
4. Basic fibroblast growth factor (bFGF; FGF-2; Specific Activity $\geq$ 2 X 10^6 Units/mg. (Catalog No. GF003)
5. Laminin (Catalog No. CC095)
6. Accutase™ (Catalog No. SCR005)
7. Tissue culture-ware
8. Glass coverslips
9. Phosphate-Buffered Saline (1X PBS) (Catalog No. BSS-1005-B)
10. Fixative (e.g. 4% Paraformaldehyde in 1X PBS)

11. Blocking Solution (5% normal donkey serum, 0.3% Triton X-100 in 1X PBS)
12. Non-Permeable Blocking Solution (5% normal donkey serum in 1X PBS)
13. Fluorescent-labeled secondary antibodies. Donkey anti-mouse IgG, Cy3 conjugated (Catalog No. AP192C), donkey anti-rabbit IgG, Cy3 conjugated (Catalog No. AP182C), and donkey anti-goat IgG, Cy3 conjugated (Catalog No. AP180C) are recommended
14. 4'-6-Diamidino-2-phenylindole (DAPI) / PBS solution
15. Anti-fading mounting solution (DABCO/PVA)
16. Hemacytometer
17. 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 human embryonic stem cells and/or human neural stem cells in the appropriate expansion medium with growth factors until the cells are 60-70% confluent.
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^{o}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 Oct-4. Nestin, Sox 2, βIII-tubulin, GAD67, ChAT, and TH. Use the

Non-Permeable Blocking Solution (5% Normal donkey serum in 1X PBS) with the antibody directed against SSEA-4.

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-Oct 4: 1/100 dilution of 1 mg/mL, final 10 μg/mL

   Mouse anti-SSEA-4: 1/100 dilution of 1 mg/mL, final 10 μg/mL

   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

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

   Rabbit anti-GAD67: 1/500 dilution of 1 mg/mL, final 2 μg/mL

   Goat anti-ChAT: 1/100 dilution of goat polyclonal antibody

   Rabbit anti-TH: 1/250 dilution of rabbit polyclonal

7. In a separate control well, depending upon the specific antibody used, add equivalent concentrations of mouse IgG, rabbit IgG or goat IgG to 0.5 mL of the appropriate blocking solution. For example, to obtain a 1/500 dilution of mouse anti-Nestin (1 mg/mL) antibody, 1 μL of the antibody is added to 0.5 mL volume of the Blocking Solution. In an adjacent control well, add 1 μL mouse IgG (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 (Catalog No. AP192C), donkey anti-mouse IgG FITC conjugated (Catalog No. AP192F), donkey anti-rabbit IgG Cy3 conjugated (Catalog No. AP182C), donkey anti-rabbit IgG FITC conjugated (Catalog No. AP182F), and donkey anti-goat IgG Cy3 conjugated (Catalog No. AP180C), and donkey anti-goat IgG FITC

conjugated (Catalog No. AP180F) antibodies at a 1:250 to 1:500 dilutions.

12. Overlay the cells with the appropriate donkey anti-mouse, anti-rabbit, and anti-goat 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.

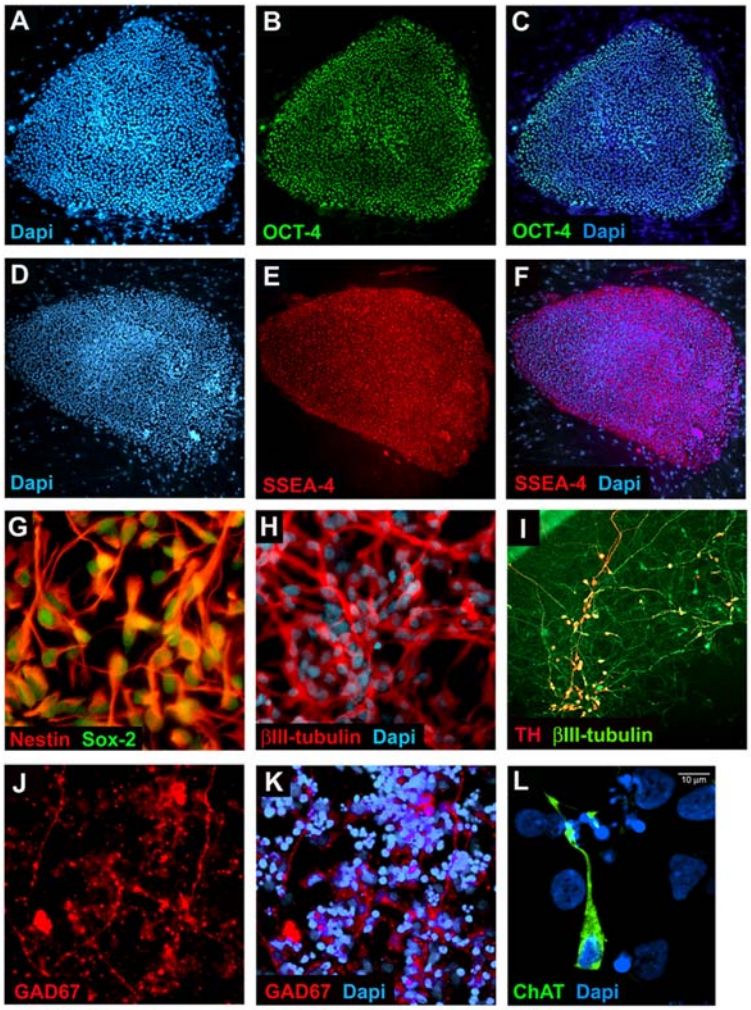
14. Counterstain the cell nuclei with DAPI / 1X PBS solution.

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 using the Human Embryonic Stem Cell Neurogenesis Characterization Kit (Catalog No. SCR065)



Characteristic staining of H9 human embryonic stem cells with pluripotent markers Oct 4 (Catalog No. MAB4401, **B**, **C**. green) and SSEA-4 (Catalog No. MAB4304, **E**, **F**, red). Oct-4 staining is nuclear and colocalizes with Dapi counter stain. ReNcell CX immortalized human cortical neural progenitor cells (Catalog No. SCC007) stained for NSC markers, Nestin (**G**, red) and Sox-2 (**G**, green). The Sox-2 transcription factor is localized to the cell nucleus. ReNcell CX cells were differentiated into neurons (βIII-tubulin; **H,** red) while ReNcell

VM (Catalog No. SCC008) can be reliably differentiated into dopaminergic neurons (TH; **I**, red). ENStem-A human neural progenitor cells (Catalog No. SCR003) can be differentiated into multiple neural cell types including GABAergic neurons (GAD67; **J**, **K**, red) and cholinergic neurons (ChAT; L, green). Nuclei of the cells were visualized with DAPI (blue).

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

References

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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.

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Related Products

The following products are available from Millipore as separate items:

1. HEScGRO Medium for Human ES Cell Culture: (Catalog No. SCM020)
2. ReNcell CX Immortalized Cell Kit: (Catalog No. SCC009)
3. ReNcell VM Immortalized Cell Kit: (Catalog No. SCC010)
4. ReNcell NSC Maintenance Media: (Catalog No. SCM005)
5. ReNcell NSC Freezing Medium: (Catalog No. SCM007)
6. ENStem-A Human Neural Progenitor Cell Kit: (Catalog No. SCR055)
7. ENStem-A Neural Expansion Medium: (Catalog No. SCM004)
8. ENStem-A Neural Differentiation Medium: (Catalog No. SCM017
9. ENStem-A Neural Freezing Medium: (Catalog No. SCM011)
10. Neuron-Glial Marker Sampler Kit: (Catalog No. NS130)
11. Embryonic Stem Cell Derived Neuron Integration and Characterization Kit: (Catalog No. NS140)
12. Dopaminergic Neuron Integration and Characterization Kit: (Catalog No. NS145)
13. Human Embryonic Germ Layer Characterization Kit: (Catalog No. SCR030)
14. Mouse anti-OCT-4, 100 µg: (Catalog No. MAB4401)
15. Mouse anti SSEA-4, 100µg: (Catalog No. MAB4304)
16. Mouse anti-Nestin, 100 µg: (Catalog No. MAB5326)
17. Rabbit anti-Sox-2, 100 µg: (Catalog No. AB5603)
18. Mouse anti-βIII tubulin, 100 µL: (Catalog No. MAB1637)
19. Rabbit anti-TH, 100 µL: (Catalog No. AB152)
20. Goat anti-ChAT, 100 µL: (Catalog No. AB144P)
21. Rabbit anti-GAD67, 100 µg: (Catalog No. AB9706)

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