HPSC Neural Induction Medium (PSCNIM-100)

Catalog #5931-100

Introduction

Human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC) possess the capability of differentiating into all derivatives of the three germ layers including neural cells. Human pluripotent stem cell (hPSC) derived neural cells have been used for studying nervous system development, modeling neurologic disorders, neurotoxicology and drug screening.

SMAD signaling plays a crucial role in neural induction. Dual inhibition of SMAD signaling by Noggin and SB431542 is sufficient to induce rapid and complete neural conversion of hPSCs [1]. In 2010, Wenlin Li and colleagues found that synergistic inhibition of glycogen synthase kinase 3 (GSK3) and transforming growth factor β (TGF-β) could efficiently differentiate hPSCs to homogenous neural epithelium in 7 days [2]. The derived neural stem cells (NSCs) can self-renew in the presence of human leukemia inhibitory factor (LIF) and inhibitors of GSK3 and TGF-β. NSCs possess the multipotential of differentiating into various neuronal and glial subtypes upon induction with specific patterning cues [2].

Based on the advances in neural differentiation methodologies, we developed hPSC Neural Induction Medium (PSCNIM, Cat. #5931), a serum-free medium, to efficiently convert hPSCs to NSCs under adherent culture conditions. The hPSCs are converted to homogenous neural epithelium using neural induction medium (Figure 1). After a 7 – 10 day treatment with PSCNIM, more than 90% of cells expressed the neural stem cell markers: Nestin and Sox2; while the pluripotency marker Nanog expression was lost (Figure 2). NSCs derived from hPSC can be maintained in the induction medium for at least 5 passages (~24 days), can efficiently form rosettes (Figure 3) and be expanded as neural progenitor cells in EGF and FGF containing medium, or differentiated into neural lineages such as neurons and astrocytes (Figure 3).

Components

Neural induction basal medium (Cat. #5931-100)	100 mL
Neural induction medium supplement 50x (Cat. #5982-100)	2 mL

Additional Materials Required

Product	Vendor and Catalog Number
BD Matrigel™ hESC-qualified matrix	BD Biosciences, Cat. #354277
StemDS® cell dissociation solution	ScienCell Research Laboratories, Cat. #5803
DPBS without Ca^{2+} and Mg^{2+}	ScienCell Research Laboratories, Cat. #0303
Y-27632 ROCK Inhibitor	Tocris Bioscience, Cat. #1254

Storage

Store the neural induction basal medium at 4°C and the neural induction medium supplement (50x) at -20°C. Protect from light.

Shipping

Neural induction medium supplement (50x) is shipped on dry ice. Neural induction basal medium is shipped at 4°C.

References

[1] Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. (2009) "Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling." *Nat Biotechnol*. 27(3): 275-280.

[2] Li W, Sun W, Zhang Y, Wei W, Ambasudhan R, Xia P, Talantova M, Lin T, Kim J, Wang X, Kim W, Lipton SA, Zhang K, Ding S. (2011) "Rapid induction and long-term self-renewal of primitive neural precursors from human embryonic stem cells by small molecule inhibitors." *PNAS*. 108(20): 8299-8304.

Instructions for use

Seeding hPSC for neural differentiation

Note: It is critical to maintain high quality, undifferentiated hPSC for differentiation. hPSCs are maintained under feeder-independent conditions. We routinely culture hPSCs in STEMium® medium (Cat. #5801) and passage the cells every 3 - 5 days. Please refer to the STEMium® medium product sheet for detailed instructions.

Note: The following procedures are optimized for 12-well plates. Indicated volumes are for one well of a 12-well plate. Please adjust volumes accordingly if using other vessels.

Note: When hPSC cultures are approximately 70 - 80% confluent, cells are ready for splitting and seeding onto 12-well plates for neural induction.

1. Prepare BD Matrigel™-coated 12-well plates in advance and warm to room temperature prior to use.
2. Warm sufficient volumes of hPSC culture medium and StemDS® cell dissociation solution (Cat. #5803) for splitting. Add Y-27632 ROCK inhibitor to the culture medium to a final concentration of 5 µM.
3. Wash cells once with 2 mL of DPBS.
4. Aspirate DPBS and gently add 2 mL of StemDS® per well. Incubate at 37°C for 8 - 10 minutes or until cells round up. After incubation, when viewed under a microscope, the majority of cells should round up but remain adherent.
5. Aspirate StemDS®.
6. Dislodge cells from plate by dispersing 1 mL of culture medium onto the cells and pipetting up and down 1 - 3 times.
7. Count the number of live cells using a hemocytometer.
8. Plate the cells onto the Matrigel coated 12-well plates in the hPSC culture medium containing 5 µM Y-27632 ROCK inhibitor. We routinely seed cells at a density of 2.5 x 10^4 cell/cm^2.

   Note: If you culture hPSCs in STEMium® medium, we recommend seeding the cells at a density of 0.1 x 10^6 cells/well in 2 mL of medium in the 12-well plate.
9. Distribute the cells evenly by moving the plates in several quick back-and-forth and side-to-side motions. Culture the cells in the incubator overnight.

   Note: Even distribution of the cells is crucial for high differentiation efficiency.

Differentiation of monolayer hPSC to neural stem cells:

Note: On the day of differentiation, the hPSCs should reach ~20% confluency.

Note: The following procedures are optimized for 12-well plates. Indicated volumes are for a single well of the 12-well plate. Please adjust volumes and cell density accordingly if scaling up.

1. **On Day 1**, thaw Neural Induction Medium Supplement (50X) and warm the basal medium to room temperature.
2. Decontaminate the external surfaces of the basal medium bottle and the supplement tube with 70% ethanol and transfer them to a sterile field.
3. Pipette up and down to mix the content in the supplement tube. Transfer the supplement to the basal medium and mix well by pipetting up and down several times.
4. Aspirate the hPSC culture medium from the cells and apply 2 mL of the induction medium to the cells.
5. Culture the cells in a 37°C, 5% CO2 with 95% humidity incubator.
6. **On Day 2 and 3**, aspirate medium from the cells and apply 2 mL of the fresh neural induction medium per well every day.

   Note: Cells usually reach 90 - 100% confluence on day 3.
7. **On Day 4 and 5**, change neural induction medium every day. Add 3 mL of the induction medium to each well.
8. **On Day 6 and 7**, change neural induction medium every day. Add 4 mL of the medium to each well.
9. **On Day 8**, more than 90% of cells should express the nestin marker at this stage. The hPSC-derived NSCs are ready to be harvested. Cells can be passaged for longer treatment or even maintained in the induction medium for at least 5 passages. Alternatively, the NSC can be expanded in EGF and FGF containing medium, or used for further differentiation.

   Note: This procedure was developed using hESC H9 and our hiPSC lines. For a specific hPSC line, some modifications may be necessary, such as longer treatment or timing of first passage.
10. A) To maintain hPSC-derived NSCs in the neural induction medium, we recommend using StemDS® or Accutase® to dissociate cells. Adding ROCKi in plating medium improves cell viability and attachment on Matrigel-coated cultureware. Seed cells at high density and a splitting ratio of 1:3 to 1:6 is recommended.

    B) To expand NSC in EGF and FGF containing medium, harvest and passage NSC following the instructions in part A. On the next day, neural tube-like rosettes can form efficiently in the EGF and FGF containing medium.

C) For differentiation, neural patterning inducers, such as retinoic acid, SHH, and FGF8, can be applied as early as day 5 of treatment.

Caution: Handling human-derived products is potentially biohazardous. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1] Grizzle WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Culture Methods*. 11: 191-9.

Troubleshooting Table

Problem	Suggestions
Poor attachment of hPSC in Matrigel™-coated plates	Seed cells in medium containing 5 µM ROCK inhibitor Y-27632.
Cell death caused by high density or cell detachment after the 7 day treatment	Split cells once with StemDS and re-plate at high density during the 7 day treatment.
Non-neural differentiation	Use high quality and undifferentiated hPSC for neural induction. Before passaging NSC, manually remove or aspirate off non-neural cell colonies which intersperse among the neural epithelium (Figure 4).

Schematic of neural induction:

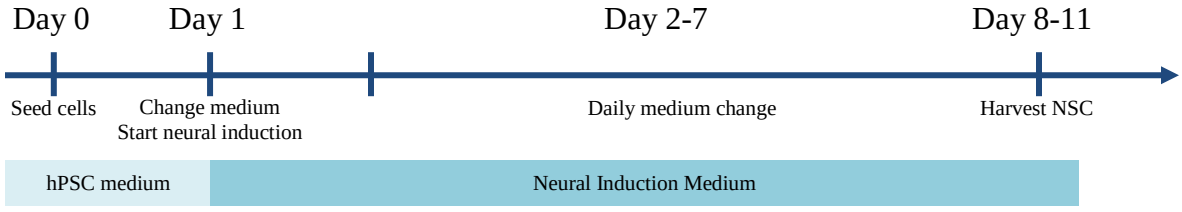
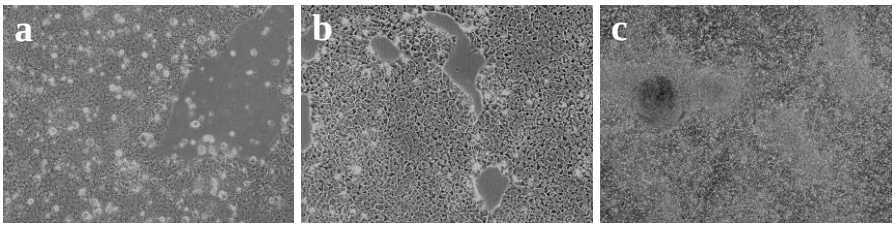
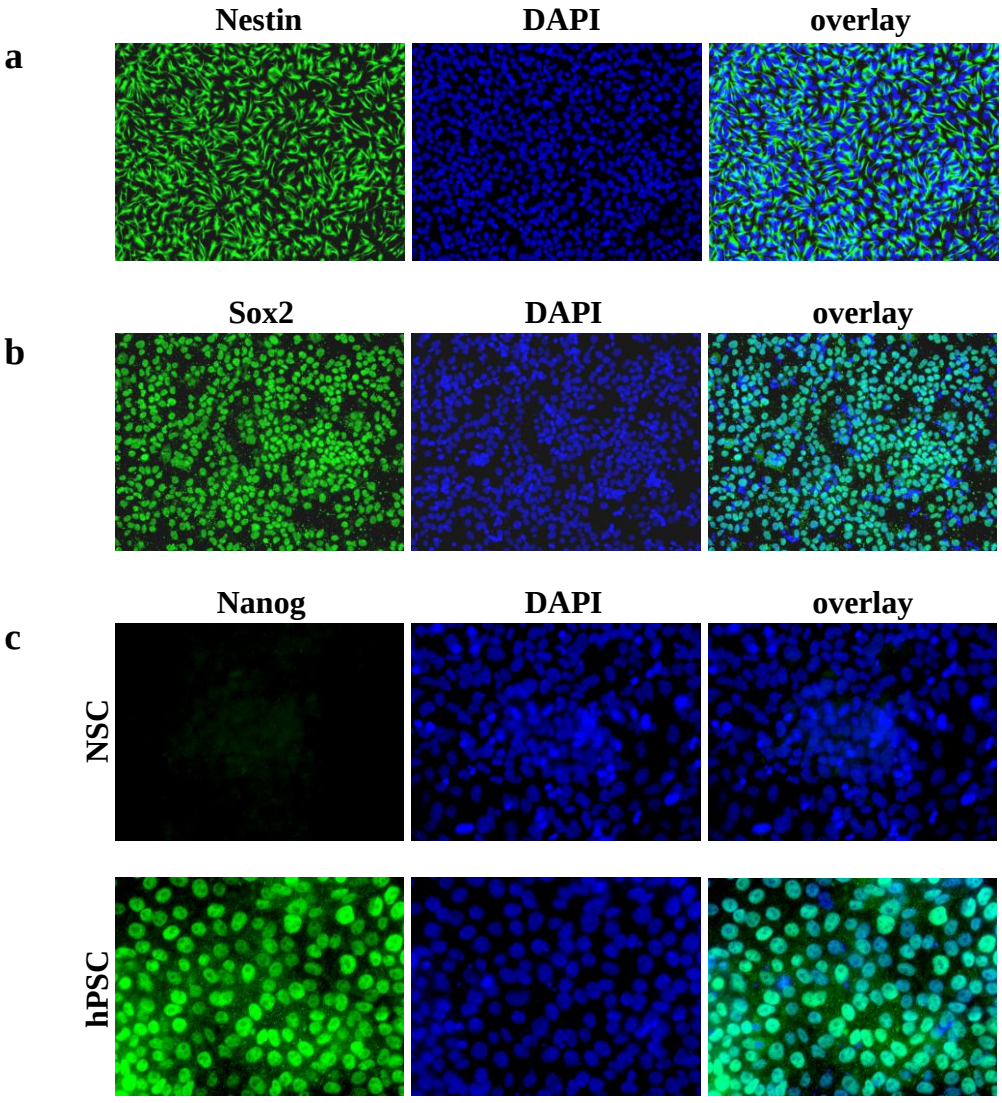


Figure 1. Converting hPSC to homogenous neural epithelium using the neural induction medium



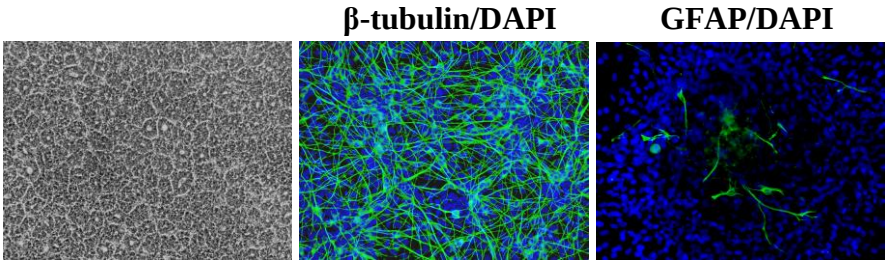
The monolayer hPSCs were cultured in the neural induction medium for 7 days and differentiated to a dense multilayer neural epithelium. a) hPSCs cultured in STEMium before treatment; b) cells treated for 2 days; c) cells cultured in the induction medium for 7 days

Figure 2. hPSC derived neural stem cells expressing Nestin and Sox2



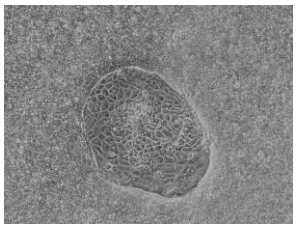
After the 7 day induction, NSCs were re-plated on Matrigel-coated coverslips for immunostaining. Cells express the NSC marker Nestin (a, green) and Sox2 (b, green), but not the hPSC marker Nanog (c, upper, green) which is expressed in hPSC (c, bottom, green). Nuclei were stained with DAPI (blue).

Figure 3. Expansion and differentiation of the hPSC-derived NSC



After splitting and seeding at high density, NSCs (P1) grow as neural-tube like structures in rhEGF and rhFGF-2 containing medium (left). Cells could differentiate into β-tubulin expressing neurons (middle, green) and GFAP expressing astroglia (right, green). Nuclei were stained with DAPI (blue).

Figure 4. Non-neural differentiation occasionally occurs with neural induction.



If the starting hPSC population is not homogenous, non-neural differentiation could occur. When differentiating a HDF-derived iPSC line to NSC, we observed non-neural differentiated cells (left), which grow as clones interspersing among the dense NSC layer and could be removed manually or by aspiration.