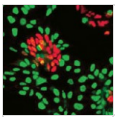

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A Monolayer Culture Method for Neural Induction of Human Pluripotent Stem Cells

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ABSTRACT

Multipotent neural progenitor cells (NPCs) generate the major cell types of the central nervous system (CNS): neurons, astrocytes and oligodendrocytes. Human pluripotent stem cells (hPSCs), including embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, can be directed to differentiate into NPCs using a variety of methods. This process, known as 'neural induction', must be efficient and reliable, in order to generate high-quality NPCs for downstream applications. Although neural induction of hPSCs using embryoid body (EB) formation allows for visual assessment of induction success via the formation of neural rosettes, the whole process can take anywhere between 16 and 19 days to get single-cell neural progenitor cells (NPCs). As a result, monolayer culture-based neural induction methods have recently gained popularity, since they enable single-cell NPCs to be obtained in as few as six days. Here we describe a procedure for neural induction using STEMdiff™ Neural Induction Medium in a monolayer culture-based system to efficiently generate PAX6-positive NPCs.

EXTERNAL LINK

https://www.stemcell.com/monolayer-culture-method-neural-induction-hpscs-lp.html?utm_source=protocolsio&utm_medium=referral

STEPS MATERIALS

NAME	CATALOG #	VENDOR
STEMdiff™ Neural Induction Medium	05835	Stemcell Technologies
Gentle Cell Dissociation Reagent	07174	Stemcell Technologies
DMEM/F-12 with 15 mM HEPES	36254	Stemcell Technologies
Y-27632	72303	Stemcell Technologies
Trypan Blue	07050	Stemcell Technologies
ACCUTASE™	07920	Stemcell Technologies
STEMdiff™ Neural Progenitor Medium	05833	Stemcell Technologies

BEFORE STARTING

This procedure is for neural induction of embryonic stem (ES) or induced pluripotent stem (iPS) cells cultured in 10 cm^2 culture dishes. If using alternative cultureware, adjust volumes accordingly. This protocol was developed using H9 human ES cells and has been validated with the H1, H9, WLS-1C, WLS-4D1, and STiPS cell lines. It may be necessary to modify some steps of the protocol, such as seeding density (step 12) or timing of first passage (step 14), to optimize performance for other cell lines.

Preparation of Materials

1 Before beginning the experiment, prepare Poly-Ornithine/Laminin- or Matrigel®-coated plates or coverslips.

Procedure for Neural Induction

2 Pre-warm STEMdiff™ Neural Induction Medium, Gentle Cell Dissociation Reagent, phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+}, and DMEM/F-12 to 37 °C

STEMdiff™ Neural Induction Medium
by Stemcell Technologies
Catalog #: 05835

Gentle Cell Dissociation Reagent
by Stemcell Technologies
Catalog #: 07174

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DMEM/F-12 with 15 mM HEPES
by Stemcell Technologies
Catalog #: 36254

3 Estimate the volume of STEMdiff™ Neural Induction Medium required for initial seeding (see Table 1), and supplement with 10 µM Y-27632 (ROCK inhibitor).

Y-27632
by Stemcell Technologies
Catalog #: 72303

Cell density is critical for achieving success. For initial plating, seed hPSCs between 200,000 and 250,000 cells/cm^2. If seeding at too low a density, the efficiency of neural induction will be reduced.

Cultureware	Volume/Well (mL)	Number of Cells Required	
		200,000 cells/cm^2	250,000 cells/cm^2
6-well plate	2.0	2.0 x 10^6	2.5 x 10^6
12-well plate	1.5	8.0 x 10^5	1.0 x 10^6
24-well plate	1.0	4.0 x 10^5	5.0 x 10^6

Table 1. Suggested volumes of medium and cell numbers required to achieve recommended seeding densities.

4 Inspect each 10 cm^2 plate of human ES or iPS cells (previously maintained in mTeSR™1 (Stemcell Technologies, catalog #85850) or TeSR™-E8™(Stemcell Technologies, catalog #05990)) and aspirate any areas of differentiated cells.

5 Rinse each plate once with 5 - 10 mL of sterile PBS.

6 Add 3 mL of Gentle Cell Dissociation Reagent and incubate at **37 °C for 8 - 10 minutes**.

7 After incubation period, gently dislodge cells that are still attached, using a 5 mL pipet. Triturate cells by pipetting up and down 5 - 10 times.

8 Add 5 mL of DMEM/F-12 and collect cells into a 50 mL conical tube.

Optional: Add an additional 5 mL of DMEM/F-12 onto the 10 cm^2 plate to rinse off any remaining cells and add to the 50 mL tube.

9 Count viable cells e.g. using Trypan Blue and a hemacytometer.

Trypan Blue
by Stemcell Technologies
Catalog #: 07050

10 Centrifuge cells **300 x g for 10 minutes**

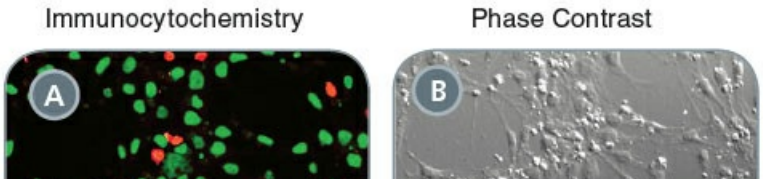
11 Resuspend cells in an appropriate volume of STEMdiff™ Neural Induction Medium supplemented with 10 µM Y-27632 to achieve a seeding density of 200,000 or 250,000 cells/cm^2, for ES cells and iPS cells respectively. See suggested volumes in Table 1.

12 Seed cells onto Poly-Ornithine/Laminin- or Matrigel®-coated plates or coverslips.

13 Replace medium daily with fresh STEMdiff™ Neural Induction Medium. Y-27632 is not required after seeding. If performing further differentiation to neuronal lineage, see Steps 15-16.

14 After six to nine days in STEMdiff™ Neural Induction Medium, cells will be OCT4-negative and PAX6-positive (Figures 1 - 2). At this point, cultures will be confluent and ready for passaging using ACCUTASE™.

ACCUTASE™
by Stemcell Technologies
Catalog #: 07920



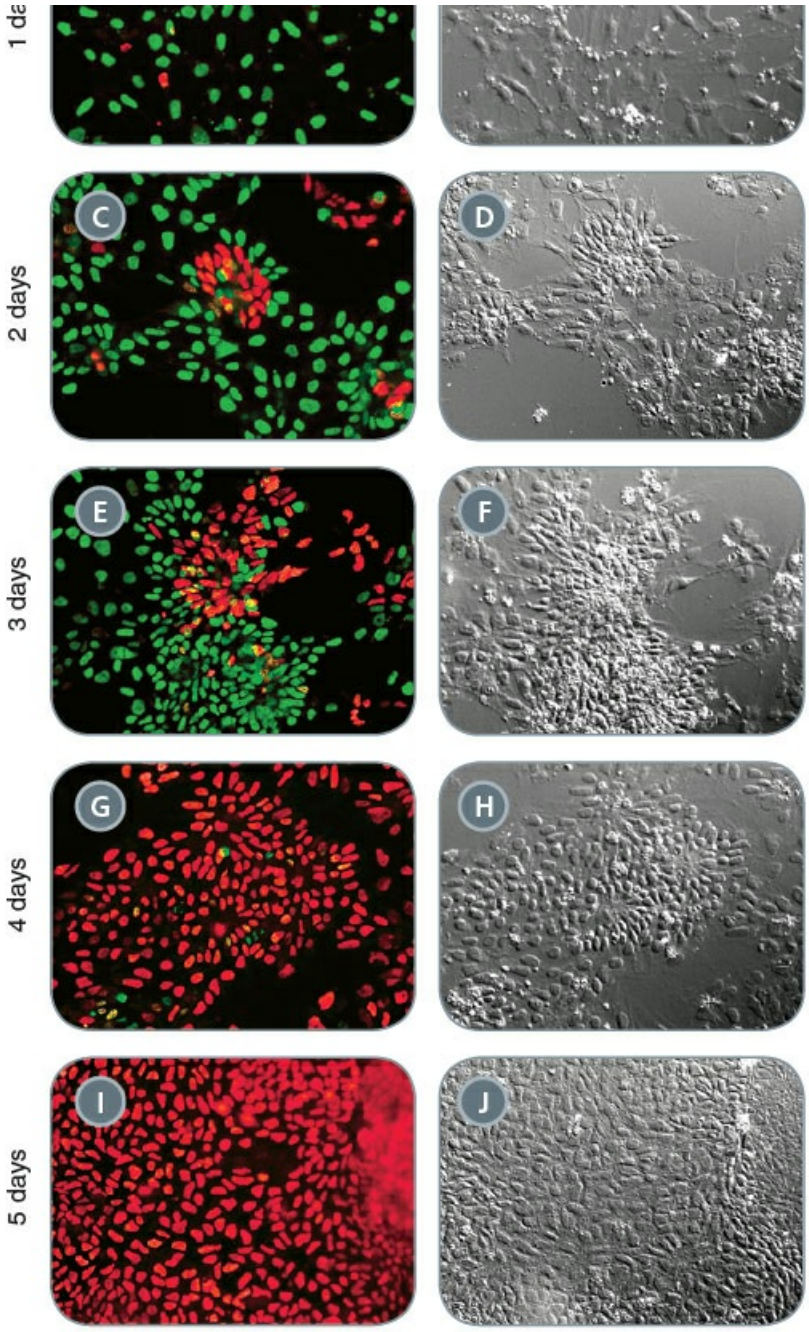


Figure 1. Immunocytochemistry and phase contrast time-course of neural induction of human iPS cells using a monolayer culture protocol.

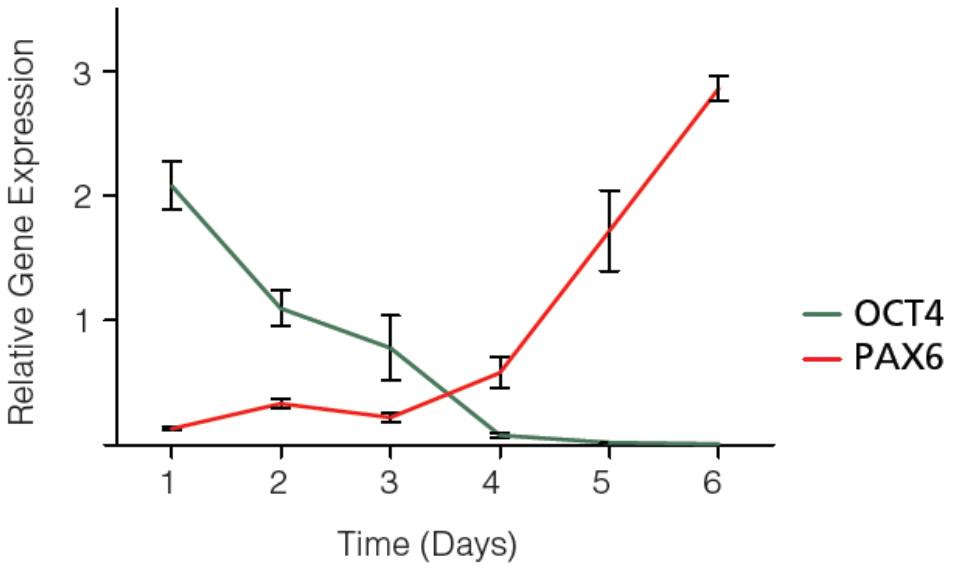


Figure 2. Downregulation of OCT4 and upregulation PAX6 during neural induction of human ES cells using a monolayer culture protocol.

Visual inspection of cultures is not a reliable method of confirming neural induction. We recommend assessing expression of markers for neural induction (PAX6) and/or undifferentiated ES or iPS cells (e.g. OCT4).

Passaging

15 Maintain neural progenitor cells (NPCs) in STEMdiff™ Neural Induction Medium until passage 3. At passage 3, transition cells into STEMdiff™ Neural Progenitor Medium.

STEMdiff™ Neural Progenitor Medium
by Stemcell Technologies
Catalog #: 05833

- After the first passage, NPCs should be passaged once they reach 70 - 80% confluency, and seeded in the next passage at a density between 125,000 and 200,000 cells/cm3.
- Supplement STEMdiff™ Neural Induction Medium with Y-27632 for the first day after each passage. Y-27632 is not required when maintaining cells in STEMdiff™ Neural Progenitor Medium.

Downstream Differentiation

16 For differentiation to neuronal subtypes (e.g. dopaminergic, motor), inductive factors are added between days five and seven. Differentiation to glial subtypes requires NPCs at passage four or later.

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