STEMdiff™ Neural System

for hPSC-Derived Neural Progenitor Cell Research

Introduction

Neural progenitor cells (NPCs) are characterized by their capacity to expand and generate the major differentiated cell types of the central nervous system (CNS): neurons, astrocytes and oligodendrocytes. Human pluripotent stem cells (hPSCs), including human embryonic stem cells (ES cells) and induced pluripotent stem cells (iPS cells), can be directed to differentiate into NPCs under specific conditions. NPCs generated in this manner constitute an invaluable research tool, with applications including the study of human nervous system development and disease, and the screening of therapeutic molecules.

To facilitate this growing research field, STEMCELL Technologies has developed a suite of products (Figure 1) specifically designed for the generation, isolation, expansion, characterization and cryopreservation of hPSC-derived NPCs.

- **Generate** NPCs from human ES and iPS cells with STEMdiff™ Neural Induction Medium
- **Isolate** CNS-type NPCs with STEMdiff™ Neural Rosette Selection Reagent
- **Expand** NPCs in culture with STEMdiff™ Neural Progenitor Medium
- **Characterize** NPCs with the STEMdiff™ Human Neural Progenitor Antibody Panel
- **Cryopreserve** NPCs with STEMdiff™ Neural Progenitor Freezing Medium

These reagents for hPSC-derived NPC research are defined and serum-free. The STEMdiff™ Neural System seamlessly integrates with STEMCELL Technologies' extensive line of media and reagents for derivation, maintenance and isolation of hPSCs.

Figure 1. The STEMdiff™ Neural System

Directed Differentiation of hPSCs to Neural Progenitor Cells

STEMdiff™ Neural Induction Medium is a defined, serum-free medium for differentiation of hPSCs into NPCs in as few as 6 days. This medium is compatible with both embryoid body and monolayer neural induction protocols (Figure 2).

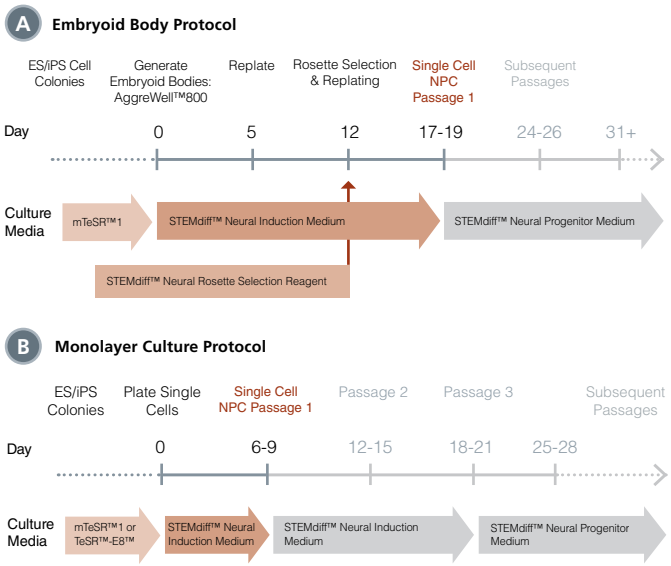

Figure 2. Schematic for Embryoid Body and Monolayer Culture Neural Induction Protocols

(A) The embryoid body (EB) protocol for neural induction using STEMdiff™ Neural Induction Medium involves EB formation, using AggreWell™800 plates, and neural rosette selection, using STEMdiff™ Neural Rosette Selection Reagent. (B) In the monolayer culture protocol, single cell hPSCs are resuspended in STEMdiff™ Neural Induction Medium and cultured as a monolayer. Following neural induction using either protocol, NPCs are expanded using STEMdiff™ Neural Progenitor Medium.

MONOLAYER PROTOCOL

A Technical Guide for Neural Induction of hPSCs Using the STEMdiff™ NIM Monolayer Protocol

www.stemcell.com/NIMmono

EMBRYOID BODY PROTOCOL

A Video Guide for Neural Induction of hPSCs Using the STEMdiff™ NIM Embryoid Body Protocol

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Neural Induction Using an Embryoid Body-Based Protocol

NPCs can be produced rapidly and efficiently from multiple human ES cell and iPS cell lines using STEMdiff™ Neural Induction Medium in conjunction with AggreWell™800 (Figure 2A). In this system, uniform embryoid bodies are first formed using AggreWell™800 plates, and are subsequently replated as adherent cultures. Morphologically distinct neural rosettes are formed in these cultures, indicative of neural induction (Figure 3A). NPCs within neural rosettes express PAX6, an early NPC marker (Figure 3B). Some of the cells at the periphery of neural rosette structures (Figure 3C) express SOX10, a neural crest marker (Figure 3D).

Neural rosette selection enables the isolation of CNS-type NPCs from a mixed culture of cells. Manual rosette selection is laborious and time-consuming. STEMdiff™ Neural Rosette Selection Reagent (Figure 4A-B) allows rapid and efficient isolation of neural rosettes without harsh enzymatic treatment. Isolated rosette clusters can be replated (Figure 4C) to generate CNS-enriched NPC cultures, which express SOX1, Nestin (Figure 4D) and PAX6, but not SOX10 (data not shown). Hereafter, NPCs can be passaged as single cells and expanded in STEMdiff™ Neural Progenitor Medium.

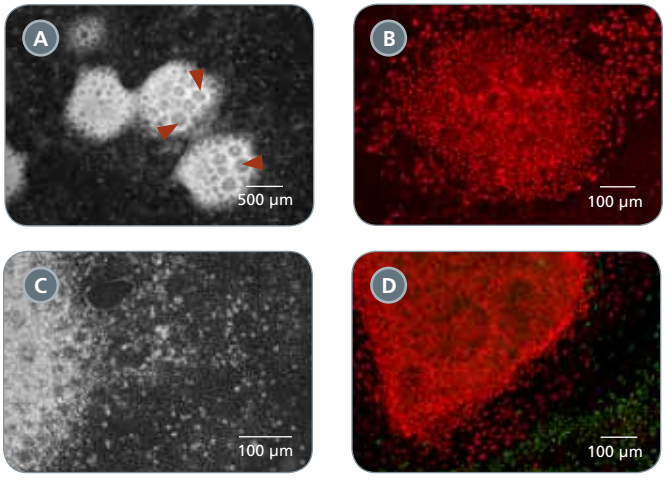

Figure 3. Morphology and Marker Expression of Neural Rosettes Generated Using STEMdiff™ Neural Induction Medium and AggreWell™800

(A) Neural rosettes (arrowheads) are clearly visible two days after replating embryoid bodies. (B) Cells within neural rosettes are PAX6$^+$ (red). (C) "Flat" cells may surround neural rosettes. (D) Some "flat" cells are neural crest-type (SOX10$^+$; green) and may surround neural rosette clusters (PAX6$^+$; red).

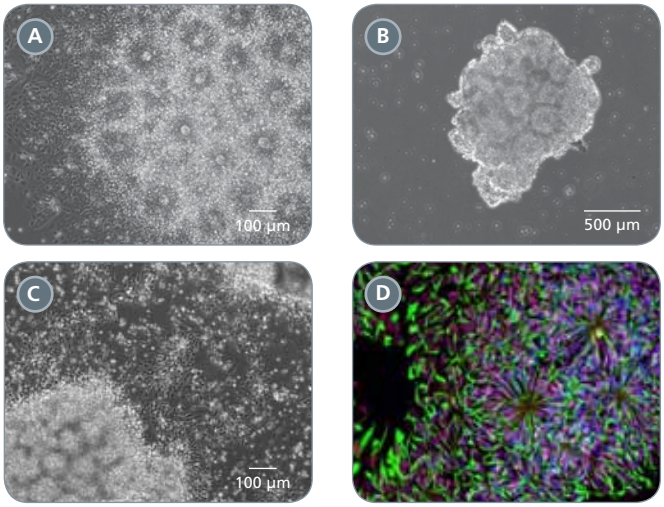

Figure 4. Selective Detachment and Replating of CNS-Type NPCs

(A) Neural rosettes after seven days on an adherent substrate, prior to neural rosette selection. (B) Neural rosette clusters selectively isolated using STEMdiff™ Neural Rosette Selection Reagent. (C-D) NPCs grow out from replated rosettes (C) to generate CNS-enriched NPC cultures that express SOX1 (D; red), Nestin (D; green) and PAX6 (not shown), but not SOX10 (not shown). Nuclei are counterstained with DAPI.

Neural Induction Using a Monolayer Culture-Based Protocol

Neural induction of hPSCs can also be achieved using a monolayer culture protocol, which does not require embryoid body formation and replating. In this protocol, hPSCs are plated as single cells in STEMdiff™ Neural Induction Medium, to generate a monolayer culture (Figure 2B). Assessment of marker expression is necessary to confirm neural induction, because cell morphology is not always a reliable indicator. By day eight, the majority of cells are PAX6-positive NPCs (Figure 5). Hereafter, NPCs can be passaged as single cells and maintained in STEMdiff™ Neural Progenitor Medium.

Advantages of STEMdiff™ Neural Induction Medium:

- Defined and serum-free
- Rapid and efficient neural induction
- Reproducible differentiation of multiple ES cell and iPS cell lines maintained in mTeSR™1
- Compatible with both embryoid body and monolayer culture protocols
- Convenient, user-friendly format and protocol

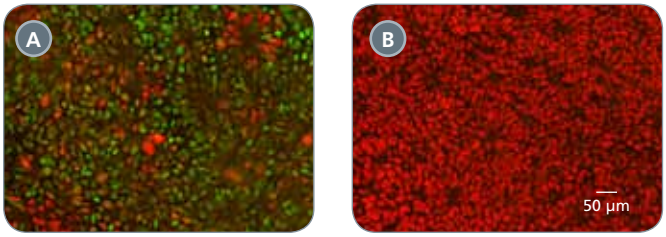

Figure 5. NPCs can be Generated Using STEMdiff™ Neural Induction Medium in a Monolayer Culture Protocol

(A) After three days in STEMdiff™ Neural Induction Medium, 25 - 30% of cells are PAX6$^+$ (red), but there are still many OCT4$^+$ (green) cells. (B) After six days, virtually all cells are PAX6$^+$/OCT4$^-$, indicating complete neural induction. A and B were taken at the same magnification.

Expansion of hPSC-Derived Neural Progenitor Cells

STEMdiff™ Neural Progenitor Medium is optimized for the expansion of NPCs generated using STEMdiff™ Neural Induction Medium. NPCs cultured in this defined and serum-free medium display typical NPC morphology and express markers that are indicative of CNS-type NPCs, such as PAX6 and SOX1 (Figure 6). These cells can be efficiently expanded in culture to generate large numbers of NPCs for downstream experiments (Figure 7). They also show minimal spontaneous neuronal differentiation (data not shown) and retain multipotency; they can differentiate further into neurons and astrocytes when directed (Figure 8).

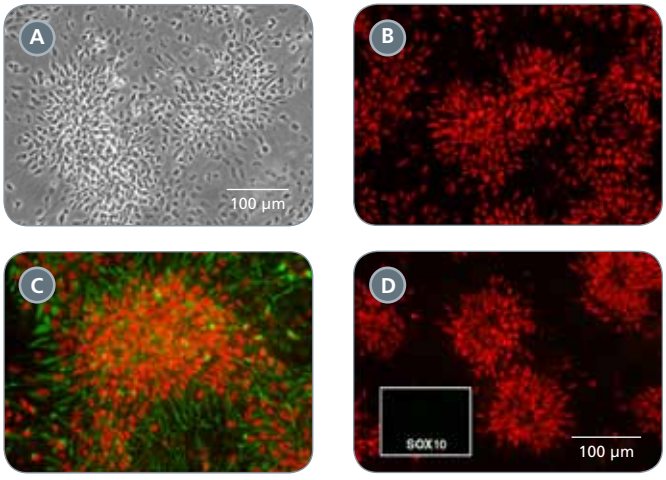

Figure 6. Morphology and Marker Expression of Neural Progenitor Cells Cultured in STEMdiff™ Neural Progenitor Medium

(A) Typical NPC morphology is observed in cultures (shown at day 6 of passage 1). (B-D) NPCs maintained in STEMdiff™ Neural Progenitor Medium express the CNS-type NPC markers PAX6 (B, D; red), SOX1 (C; red) and NESTIN (C; green), but not the neural crest marker SOX10 (D; green, single channel shown in inset). B-D were taken at the same magnification.

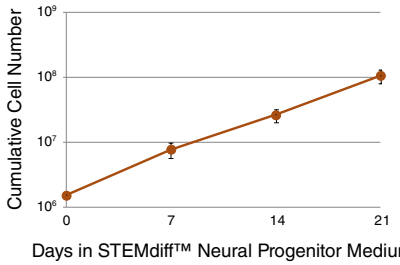

Figure 7. Expansion of Neural Progenitor Cells in STEMdiff™ Neural Progenitor Medium

NPCs cultured in STEMdiff™ Neural Progenitor Medium can be expanded to generate a large number of cells. Three- to five-fold expansion can be achieved upon each passage. NPCs were derived using STEMdiff™ Neural Induction Medium and passaged once a week on average. n = 6.

Advantages of STEMdiff™ Neural Progenitor Medium:

- Defined and serum-free
- Supports expansion of NPCs generated using STEMdiff™ Neural Induction Medium
- Optimized for efficient expansion of NPCs over multiple passages
- Preserves NPC multipotency while minimizing spontaneous neuronal differentiation
- Convenient, user-friendly format and protocol

Differentiation of hPSC-Derived Neural Progenitor Cells

NPCs generated in STEMdiff™ Neural Induction Medium are multipotent and can differentiate into neurons and glia when directed (Figure 8). In order to differentiate NPCs into diverse neuronal subtypes, NPCs must be regionalized at an early timepoint (eg. day five to nine of neural induction) using inductive signals. Dorso-ventral patterning of the developing embryonic CNS is accomplished primarily through the opposing actions of bone morphogenic proteins (BMPs) and sonic hedgehog (Shh). Shh activators (eg. purmorphamine) and inhibitors (eg. cyclopamine) can specify NPCs towards ventral or dorsal fates, respectively (Figure 9). Similarly, NPCs can be patterned along the anterior-posterior axis using organizer or posteriorizing factors, such as FGF8 or retinoic acid, respectively. NPCs generated using STEMdiff™ Neural Induction Medium are capable of responding to patterning cues, in order to generate specific neuronal subtypes. For example, adding SHH and FGF-8 to NPCs shortly

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after initiation of neural induction can further direct these cells to the dopaminergic neuronal lineage (Figure 10).

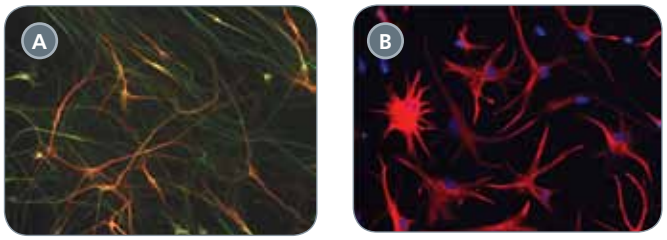

Figure 8. Neural Progenitor Cells Maintained in STEMdiff™ Neural Progenitor Medium can Differentiate into Neurons and Astrocytes

When directed according to published protocols, NPCs can differentiate into neurons (A; class III β-tubulin shown in red) and astrocytes (B; GFAP shown in red). Nuclei are counterstained with DAPI (blue).

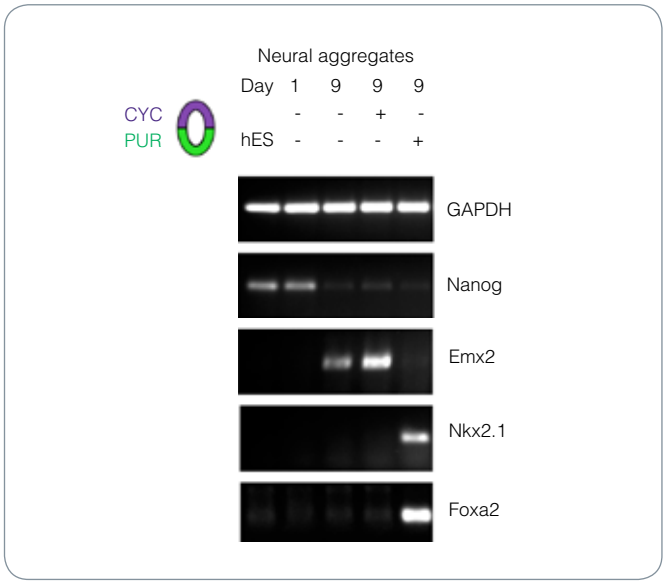

Figure 9. Dorso-Ventral Patterning of Neural Progenitor Cells

Neural aggregates were formed from H9 hES cells using AggreWell™ and STEMdiff™ Neural Induction Medium. After five days, aggregates were replated in STEMdiff™ Neural Induction Medium in the presence of cyclopamine or purmorphamine. The pluripotency gene Nanog is expressed in hES cells and day 1 neural aggregates. By day 9, very faint expression is detected. After addition of the SHH antagonist cyclopamine (CYC), expression of the dorsal forebrain marker EMX2 is increased slightly. Addition of the SHH activator purmorphamine induces the expression of the ventral markers NKX2.1 and FOXA2, and strongly represses the expression of EMX2. Data courtesy of Dr. Kim and Dr. Ghosh UCSD, San Diego, USA.

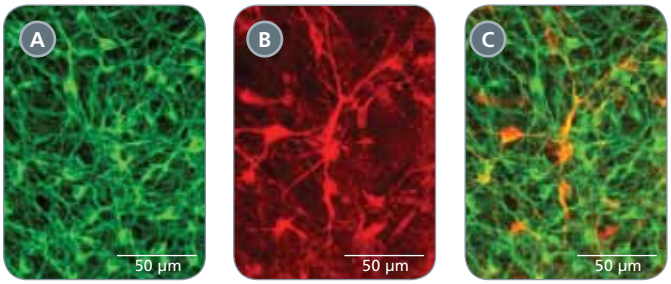

Figure 10. Differentiation of Neural Progenitor Cells to Dopaminergic Neurons

Embryoid bodies formed in STEMdiff™ Neural Induction Medium were treated with Shh (200 ng/mL) and FGF-8 (100 ng/mL) from day four of neural induction onwards. Regionalized NPCs were differentiated to dopaminergic neurons using a procedure modified from Yan et al. (Stem Cells 23: 781-790, 2005). Clusters of neurons (A, C; class III β-tubulinTUJ-1; green) are interspersed with tyrosine hydroxylase-positive (B,C; red) dopaminergic neurons. Data courtesy of Dr. Sonntag, McLean Hospital, Belmont, USA.

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www.stemcell.com/NeuroModeling

Table 1. STEMdiff™ Reagents for Neural Progenitor Cell Generation, Maintenance, Characterization and Cryopreservation

PRODUCT	SIZE	CATALOG #
STEMdiff™ Neural Induction Medium	250 mL	05835
STEMdiff™ Neural Rosette Selection Reagent	100 mL	05832
STEMdiff™ Neural Progenitor Medium	500 mL*	05833
STEMdiff™ Neural Progenitor Freezing Medium	100 mL	05838
STEMdiff™ Human Neural Progenitor Antibody Panel	1 Kit	69001

*Kit includes 50X and 1000X supplements

Table 2. Support Products for Human Pluripotent Stem Cell-Derived Neural Progenitor Cell Research

PRODUCT	CATALOG #
TeSR™-E7 Reprogramming Medium	05910
mTeSR™1 Maintenance Medium	05850 / 05870 / 05875 / 05857
TeSR™-E8™ Maintenance Medium	05940
AggreWell™800 plates	27865 / 27965