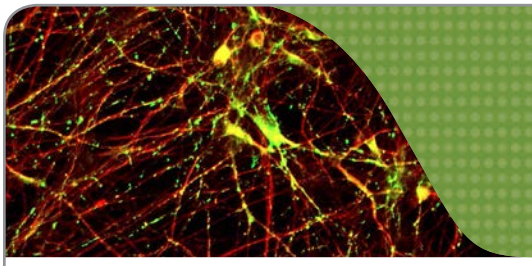

TECHNICAL BULLETIN

Neuronal Differentiation of hPSC-Derived Neural Progenitor Cells

Background

Human pluripotent stem cells (hPSCs), including embryonic stem (ES) and induced pluripotent stem (iPS) cells, can undergo neural induction to generate neural progenitor cells (NPCs). Highly enriched cultures of CNS-type NPCs may be obtained through the use of standard monolayer culture or embryoid body (EB) protocols. Further differentiation of NPCs into neurons and glia provides a physiologically relevant model for neurological disease research, drug discovery and cell therapy validation. Many published protocols for neuronal differentiation use Brewer's B27[1] supplement and/or Bottenstein's N2[2] supplement with a basal medium of choice. While traditional neuronal basal media support cell survival, they impair neurological activities, including action potential generation and synaptic activity. BrainPhys™ was designed by Dr. Cedric Bardy in Dr. Fred H. Gage's laboratory to better support in vitro neuronal function.[3] In this technical bulletin, we describe a method (based on Yuan et al.[4]) for neuronal differentiation (Figure 1), using BrainPhys™ Neuronal Medium as a basal medium, supplemented with NeuroCult™ SM1, N2 Supplement-A and other factors. Using this protocol, forebrain-type neurons can be generated from NPCs in 2 - 4 weeks. Further culture in BrainPhys™ Neuronal Medium results in cultures that are phenotypically mature and, after 65 days in culture, synaptically active.

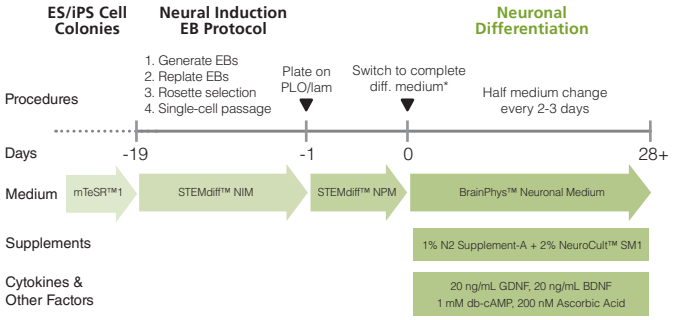

Figure 1. Schematic for Neural Induction and Differentiation Protocol

The embryoid body (EB) protocol for neural induction using STEMdiff™ Neural Induction Medium includes EB formation using Aggrewell™800 plates and neural rosette selection using STEMdiff™ Neural Rosette Selection Reagent (see full protocol in Document #28782). The neuronal differentiation protocol uses BrainPhys™ Neuronal Medium with NeuroCult™ SM1 Supplement, N2 Supplement-A and other cytokines and growth factors.

**The timing of the switch between STEMdiff™ Neural Progenitor Medium and the complete differentiation medium (Day 0) may vary from 1 - 3 days depending on cell density.*

Results

When NPCs are cultured in BrainPhys™ Neuronal Medium with NeuroCult™ SM1 and N2 Supplement-A, a mixed population of forebrain-type neurons is generated. A 2 - 4 week culture period is required for the cells to differentiate and for neuronal morphology to become apparent (Figure 2A). Further maturation under the same conditions results in neurons showing high expression of the pre-synaptic marker Synapsin 1 (Figure 2B). After 65 days of maturation, neurons show increased frequency and amplitude of spontaneous excitatory and inhibitory channel currents, compared to cultures differentiated using a traditional basal medium (see data in Document #27010). To evaluate neuronal differentiation, the markers Class III β–Tubulin, Microtubule-Associated Protein 2 (MAP2), Gamma Aminobutyric Acid (GABA), etc. can be used.

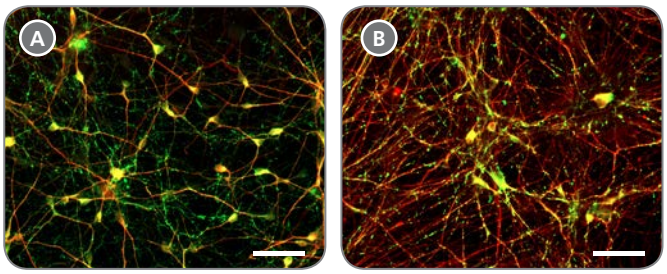

Figure 2. Forebrain-Type Neurons Generated from hPSC-Derived NPCs Using BrainPhys™ Neuronal Medium, NeuroCult™ SM1 and N2 Supplement-A

Neural induction of H9 cells was performed using STEMdiff™ Neural Induction Medium in an embryoid body (EB)-based protocol. (A-B) After neural induction, cells were cultured in BrainPhys™ Neuronal Medium with 2% NeuroCult™ SM1 Supplement, 1% N2 Supplement-A and other factors to initiate neuronal differentiation. After (A) 14 and (B) 44 days, neurons have developed long processes and express Synapsin 1 (green), MAP2 (A; red) and Class III β-Tubulin (B; red). Scale bar 50 µm.

Protocol: Neuronal Differentiation of hPSC-Derived Neural Progenitor Cells

This protocol describes a method for neuronal differentiation of NPCs derived from H9 hPSCs.[4] Some protocol optimization may be required for cells derived from other cell lines.

Generation of Neural Progenitor Cells

Generate NPCs using the EB protocol. For complete instructions, refer to the Technical Manual: Generation and Culture of Neural Progenitor Cells using the STEMdiff™ Neural

Neuronal Differentiation of hPSC-Derived Neural Progenitor Cells

System (Document #28782). After the first single-cell passage onto poly-L-ornithine/laminin-coated dishes (15 µg/mL and 10 µg/mL, respectively), culture NPCs in STEMdiff™ Neural Progenitor Medium for 1-3 days, until 25 - 50% confluent, replenishing the medium daily.

Procedure for Neuronal Differentiation

1. Prepare complete differentiation medium:
    - Basal medium: BrainPhys™ Neuronal Medium
    - Supplements (working concentrations)
        - 2% NeuroCult™ SM1
        - 1% N2 Supplement-A
        - 20 ng/mL BDNF
        - 20 ng/mL GDNF
        - 1 mM dibutyryl cAMP
        - 200 nM Ascorbic Acid
2. When cells have reached 25 - 50% confluency, add an equal volume of complete differentiation medium to the existing medium in each well.
3. Perform a half-medium change every 2 - 3 days by removing ~50% of the culture medium from each well and replacing with an equal volume of fresh complete differentiation medium. A 2 - 4 week culture period will be required for cells to differentiate and for mature neuronal morphology to become apparent.
4. Analyze for neuronal differentiation using markers such as Class III β-Tubulin, MAP2, GABA.

Additional Reagents

anti-Class III β-Tubulin Mouse IgG2a (Catalog #01409)

anti-MAP2 Rabbit IgG (Catalog #01410)

anti-GABA Rabbit IgG (Sigma-Aldrich; Catalog #A2052)

Important Notes

- If using the monolayer protocol for neural induction, cells should be moved into STEMdiff™ Neural Progenitor Medium at passage 3. Then, culture cells in complete STEMdiff™ Neural Progenitor Medium for 1 - 3 days at 37°C, until cells are 50% confluent, replenishing medium daily, and proceed with the neuronal differentiation protocol.
- The key optimization step is the seeding density of NPCs.

Product Information

Table 1. Materials for NPC Passaging

PRODUCT	SIZE	CATALOG #
STEMdiff™ Neural Induction Medium	250 mL	05835
STEMdiff™ Neural Progenitor Medium	500 mL kit	05833
DMEM/F-12 with 15 mM HEPES	500 mL	36254
ACCUTASE™	100 mL	07920

Table 2. Materials for Neuronal Differentiation

PRODUCT	SIZE	SUPPLIER; CATALOG #
BrainPhys™ Neuronal Medium	500 mL	05790
Poly-L-ornithine solution	50 mL	Sigma-Aldrich; P4957
Laminin	1 mg	Sigma-Aldrich; L2020
NeuroCult™ SM1 Neuronal Supplement	10 mL	05711
N2 Supplement-A	5 mL	07152
Dibutyryl cyclic-AMP sodium salt	Various	Sigma-Aldrich; D0627
Recombinant Human GDNF	Various	PeproTech; 450-10
Recombinant Human BDNF	Various	PeproTech; 450-02
Ascorbic Acid	100 mg	07157

References

1. Brewer GJ et al. (1993) Optimized survival of hippocampal neurons in B27-supplemented Neurobasal, a new serum-free medium combination. J Neurosci Res. 35(5):567-76.
2. Bottenstein JE. (1985) Cell Culture in the Neurosciences. (Bottenstein JE, Harvey A., Eds.). Plenum Press: New York and London.
3. Bardy C et al. (2015) Neuronal medium that supports basic synaptic functions and activity of human neurons in vitro. Proc Natl Acad Sci 112 (20) E2725-E2734.
4. Yuan SH et al. (2011) Cell-surface marker signatures for the isolation of neural stem cells, glia and neurons derived from human pluripotent stem cells. PLoS One 6(3):e17540.