

BrainPhys™ Neuronal Medium

For Improved Neuronal Function

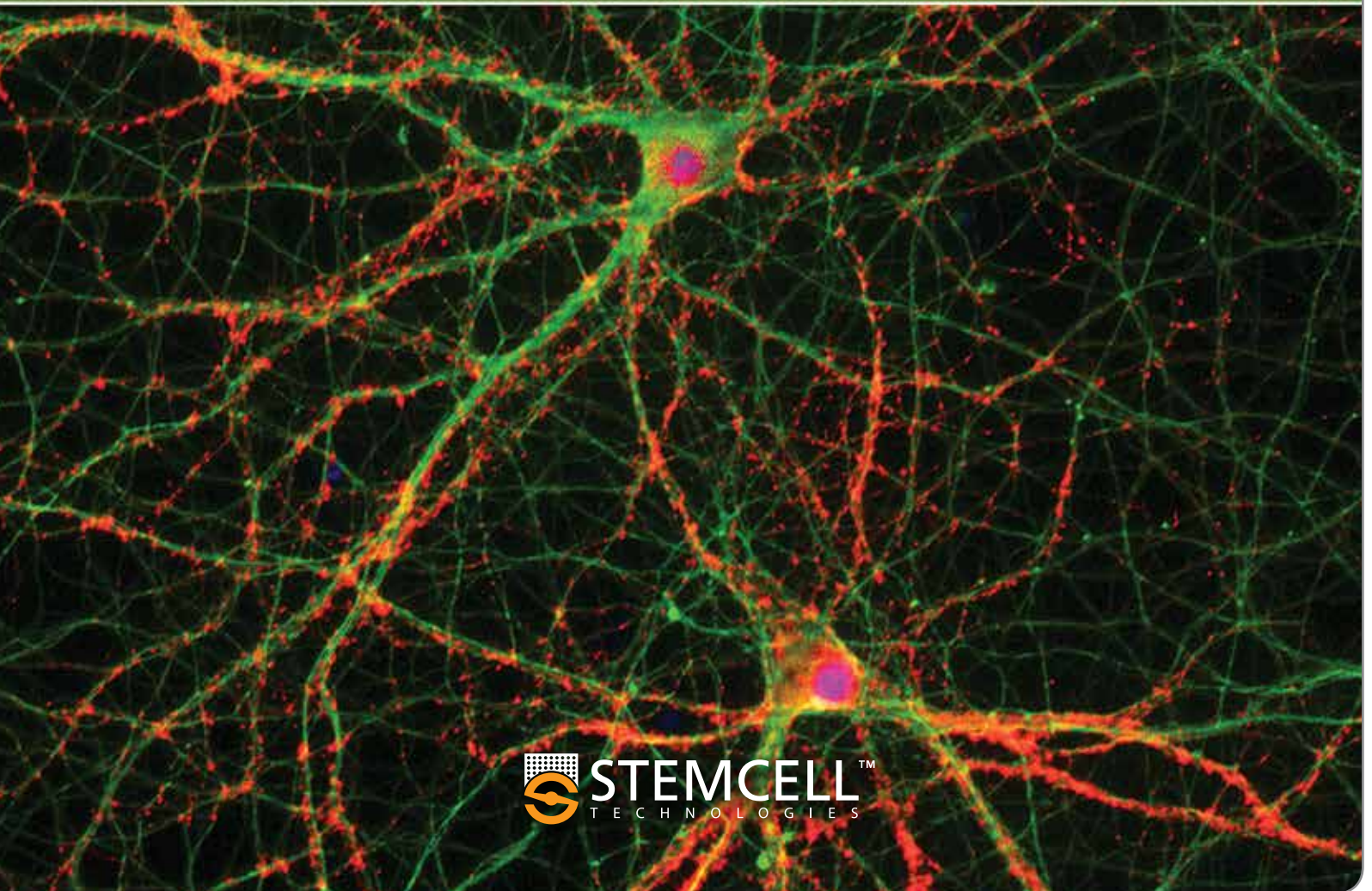


Table of Contents

- 3 Introduction
- 4 BrainPhys™ Neuronal Medium for Primary Neuronal Culture
- 6 BrainPhys™ Neuronal Medium for hPSC-Derived Neuronal Culture
- 7 Product Information
- 7 Supplementary Reagents

Scientists Helping Scientists™

STEMCELL Technologies has been setting standards for quality cell culture media and reagents since 1993. As Scientists Helping Scientists, we have developed over 1500 specialty products for stem cells isolated from various tissues. BrainPhys™ Neuronal Medium is a new neuronal basal medium for the culture of primary and hPSC-derived neurons, based on the formulation invented by Dr. Cedric Bardy and Dr. Fred H. Gage.¹ Learn more about BrainPhys™ Neuronal Medium at www.BrainPhys.com.

BrainPhys™ Neuronal Medium and Supplement Kits

Obtain optimal neuronal functional and survival of primary or hPSC-derived neurons. Kits include BrainPhys™ Neuronal Medium and NeuroCult™ SM1 Neuronal Supplement with or without N2 Supplement-A.

Front cover. Rat E18 cortical neurons cultured in BrainPhys™ Neuronal Medium for 21 days in vitro.

Introduction

BrainPhys™ Neuronal Medium is a new, defined and serum-free neuronal culture medium for the culture of both primary neurons and neurons derived from human pluripotent stem cells (hPSCs); including embryonic stem (ES) and induced pluripotent stem (iPS) cells. Traditional neuronal culture media, such as Neurobasal Medium and Dulbecco's Modified Eagle Medium (DMEM) support cell survival, but impair neurological activities, including action potential generation and synaptic activity (Table 1).¹ BrainPhys™ was designed by Dr. Cedric Bardy in Dr. Fred H. Gage's laboratory to better support in vitro neuronal function.¹ Neuronal cultures matured in BrainPhys™ Neuronal Medium experience central nervous system (CNS)-like physiological conditions and develop a higher proportion of synaptically active neurons. Furthermore, using BrainPhys™ Neuronal Medium increases the physiological relevance of in vitro neuroscience research, thus increasing the likelihood of successfully translating scientific discoveries from the laboratory to the clinic.

As a versatile neuronal basal medium, BrainPhys™ Neuronal Medium may be used with many diverse neuronal culture and differentiation protocols, in combination with appropriate neuronal supplements, cytokines or small molecules. Because BrainPhys™ Neuronal Medium supports neuronal function, assays of neuronal activity (such as calcium imaging, microelectrode array (MEA)-based recording and optogenetics) can be performed within the established cultures, without the need to replace media. This streamlines experimental workflows and minimizes sudden or frequent changes to the cultures.

Applications of BrainPhys™ Neuronal Medium

- Culture of primary mouse or rat neurons
- Differentiation and maturation of human ES/iPS cell-derived neurons
- Microelectrode array-based recording of neuronal activity
- Fluorescence-based live imaging, including calcium imaging and optogenetic stimulation and recording
- Transdifferentiation (lineage conversion) of somatic cells to neurons¹

Advantages

PHYSIOLOGICAL. More representative of the brain's extracellular environment.

ACTIVE. Improved neuronal function and a higher proportion of synaptically active neurons.

STREAMLINED. Perform functional assays without replacing media.

VERSATILE. Supports long-term culture of ES/iPS cell-and CNS-derived neurons.

RELIABLE. Rigorous raw material screening and quality control ensure minimal lot-to-lot variability.

Table 1. Properties of Culture Media¹

PROPERTY	DMEM/F12	NEUROBASAL	BRAINPHYS™
Properties of the medium			
Inorganic salt concentration	✓	Not physiological	✓
Glucose level	Hyperglycemic	Hyperglycemic	✓
Osmolarity	✓	Low	✓
Neuronal function			
Spontaneous and evoked action potentials	Impaired	Impaired	✓
Spontaneous network calcium dynamics	Impaired	<i>not tested</i>	✓
Excitatory synaptic activity	Blocked	Low	✓
Inhibitory synaptic activity	Blocked	Blocked	✓

Check-mark denotes physiological conditions and supported activities according to Bardy et al.¹

Primary Neuronal Culture

Primary neuronal cultures have long been a powerful system with which to study neuronal biology in a controlled environment. To obtain healthy cultures with good morphology and cellular function, the use of high-quality media and supplements is crucial. For optimal neuronal function and survival, plate mouse or rat neurons in a traditional neuronal medium (e.g. NeuroCult™ Neuronal Basal Medium, supplemented with NeuroCult™ SM1 Neuronal Supplement) and transition cultures to BrainPhys™ Neuronal Medium, supplemented with NeuroCult™ SM1, after 5 days in vitro (DIV).

After 14 and 21 DIV, cultures matured in BrainPhys™ Neuronal Medium show large numbers of viable neurons, with minimal cell clumping and extensive neurite outgrowth and branching (Figure 1A,C). Neuronal morphology is consistent with neurons cultured in a traditional neuronal medium (Figure 1B,D). The number of viable neurons after 21 DIV is consistently greater in BrainPhys™ Neuronal Medium-matured cultures, regardless of whether NeuroCult™ Neuronal Basal Medium or Neurobasal Medium is used as the initial plating medium (Figure 2).

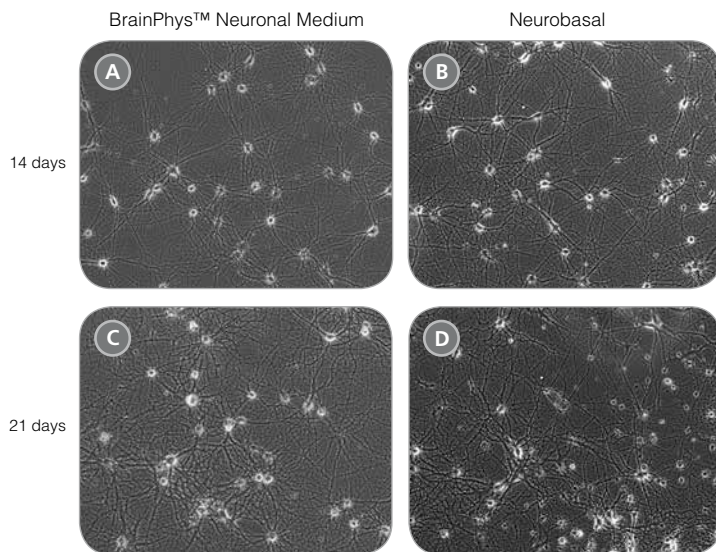


Figure 1. Rodent Neurons Matured in BrainPhys™ Neuronal Medium are Healthy and Morphologically Mature

(A,C) Primary rat E18 cortical neurons were plated in NeuroCult™ Neuronal Basal Medium, supplemented with NeuroCult™ SM1 Neuronal Supplement. After 5 DIV, the cultures were transitioned to BrainPhys™ Neuronal Medium, supplemented with NeuroCult™ SM1, by performing half-medium changes every 3 - 4 days. Neurons were cultured for 14 (A) or 21 (C) DIV. (B,D) Primary rat E18 cortical neurons were plated and matured in a traditional neuronal medium (Neurobasal Medium), supplemented with NeuroCult™ SM1 Neuronal Supplement for 14 (B) or 21 (D) DIV. Neuronal morphology of BrainPhys™ Neuronal Medium-matured neurons is consistent with neurons plated and matured in a traditional neuronal medium.

Neurons cultured in BrainPhys™ Neuronal Medium are functionally mature, and show improved synaptic activity compared to those cultured in a traditional neuronal medium (Figure 3). The frequency and amplitude of spontaneous excitatory (AMPA receptor-mediated) and inhibitory (GABA receptor-mediated) synaptic currents are increased in BrainPhys™ Neuronal Medium-matured cultures. Furthermore, using a MEA system (Axion Biosystems), the mean firing rate and percentage of active electrodes of neurons cultured in BrainPhys™ Neuronal Medium increased markedly over time, whereas both remained low in neurons cultured in a traditional neuronal medium (TNM) (Figure 4). Marker analysis confirms that neurons cultured in BrainPhys™ Neuronal Medium are phenotypically mature, as indicated by the highly branched morphology and appropriate expression of MAP2 and Synapsin 1 (Figure 5).

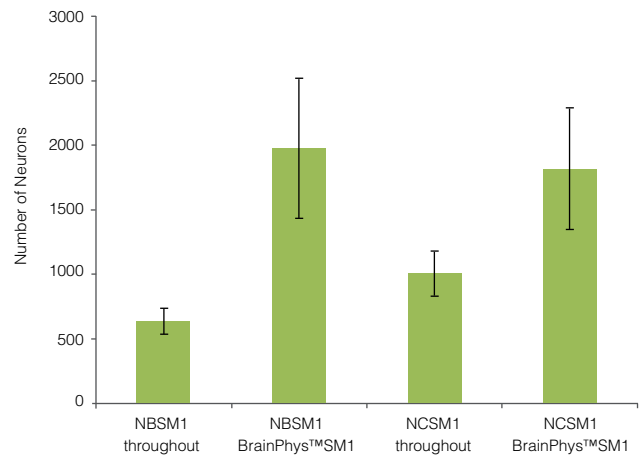


Figure 2. Primary Neuronal Cultures Matured in BrainPhys™ Neuronal Medium Have Greater Numbers of Neurons

Primary rat E18 cortical neurons were plated in either NeuroCult™ Neuronal Basal Medium (NCSM1) or Neurobasal Medium (NBSM1), supplemented with NeuroCult™ SM1. After 5 DIV, half of the cultures were transitioned to BrainPhys™ Neuronal Medium, supplemented with NeuroCult™ SM1, by performing half-medium changes every 3 - 4 days. The other half of the cultures were maintained in the same medium as used for plating. After 21 DIV, more neurons were evident in the cultures matured in BrainPhys™ Neuronal Medium, regardless of whether NeuroCult™ Neuronal Basal Medium or Neurobasal Medium was used as the plating medium. (n = 2, mean ± SEM [triplicate wells were set up for each experiment]).

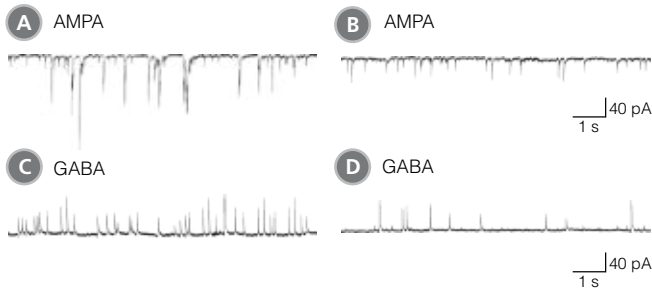


Figure 3. Rodent Neuronal Cultures Matured in BrainPhys™ Neuronal Medium Show Improved Excitatory and Inhibitory Synaptic Activity in Classic Electrophysiology Assays

(A,C) Primary rat E18 cortical neurons were plated in NeuroCult™ Neuronal Basal Medium, supplemented with NeuroCult™ SM1 Neuronal Supplement. After 5 DIV, the cultures were transitioned to BrainPhys™ Neuronal Medium, supplemented with NeuroCult™ SM1 Neuronal Supplement, by performing half-medium changes every 3 - 4 days. Neurons were cultured for 21 DIV. (B,D) Primary rat E18 cortical neurons were plated and matured in a traditional neuronal medium (Neurobasal Medium), supplemented with NeuroCult™ SM1 Neuronal Supplement for 21 DIV. (A,C) Neurons matured in BrainPhys™ Neuronal Medium showed spontaneous excitatory (AMPA-mediated; A) and inhibitory (GABA-mediated; C) synaptic events. The frequency and amplitude of spontaneous synaptic events is consistently greater in neuronal cultures matured in BrainPhys™ Neuronal Medium, compared to neurons plated and matured in a traditional neuronal medium (B,D). Traces are representative.

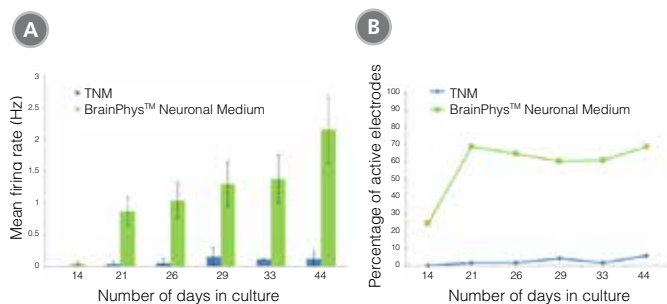


Figure 4. Primary Neuronal Cultures Matured in BrainPhys™ Neuronal Medium Show Improved Electrical Activity in Microelectrode-Array Systems

Primary rat E18 cortical neurons were plated in a traditional neuronal medium (TNM; Neurobasal Medium supplemented with NeuroCult™ SM1). After 5 DIV, half of the cultures were transitioned to BrainPhys™ Neuronal Medium, supplemented with NeuroCult™ SM1, by performing half-medium changes every 3 - 4 days. The other half of the cultures were maintained in TNM throughout. The electrical activities of the neuronal cultures were measured twice a week using a microelectrode array (MEA) system (Axion Biosystems). (A) The mean firing rate of neurons cultured in BrainPhys™ Neuronal Medium increased over time, whereas the mean firing rate of neurons in the TNM condition remained low over time ($n=1$; mean \pm SEM, 128 electrodes). (B) The percentage of active electrodes (>0.005 Hz) of neurons matured in BrainPhys™ Neuronal Medium increased from 24% on day 14 to 69% on day 21, and then remained stable at 60 - 70% from days 21 - 44. In contrast, $< 5\%$ of electrodes were active in the TNM condition over the same 6-week period.

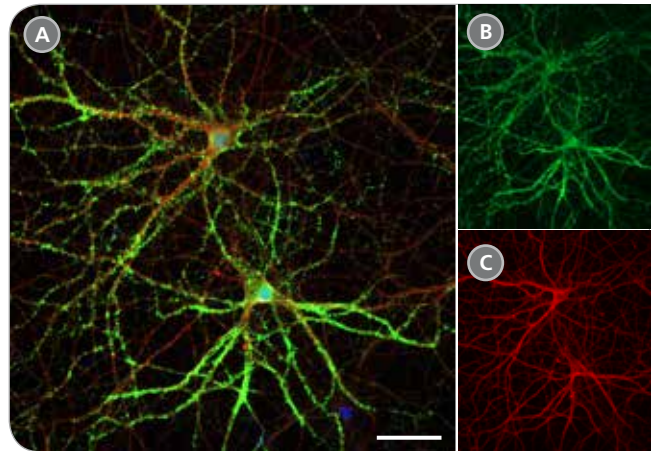


Figure 5. Expression of Pre-Synaptic Markers in Rodent Neurons Matured in BrainPhys™ Neuronal Medium

Primary rat E18 cortical neurons were plated in NeuroCult™ Neuronal Basal Medium, supplemented with NeuroCult™ SM1 Neuronal Supplement. After 5 DIV, the cultures were transitioned to BrainPhys™ Neuronal Medium, supplemented with NeuroCult™ SM1 Neuronal Supplement, by performing half-medium changes every 3 - 4 days. Neurons cultured for 21 DIV are phenotypically mature, as indicated by the presence of an extensive dendritic arbor. The pre-synaptic marker synapsin (A,B; green) is concentrated in discrete puncta distributed along the somata and dendritic processes, as defined by the dendritic marker MAP2 (A,C; red). Scale bar = 50 μ m.



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hPSC-Derived Neuronal Culture

hPSCs, including ES and iPS cells, are a valuable tool for researchers aiming to model human neurological development and disease. By generating iPS cells from patient samples, disease- and patient-specific neural stem cells, neurons and glia can be obtained. Published protocols are available for the generation of many different neuronal subtypes, using a basal medium together with neural supplements, such as NeuroCult™ SM1 Neuronal Supplement (based on the published B27 formulation²) and N2 supplement,³ and various cytokines and small molecules. Using BrainPhys™ Neuronal Medium as the basal medium for hPSC-derived neural progenitor cell (NPC) differentiation and neuronal maturation will generate a more neurophysiologically active culture that better represents the human brain environment.¹ BrainPhys™ Neuronal Medium may also be used for direct lineage conversion of somatic cells into neurons, without transitioning through a hPSC intermediate.¹

Neurons can be generated efficiently from hPSC-derived NPCs using BrainPhys™ Neuronal Medium supplemented with NeuroCult™ SM1, N2 Supplement-A, glial cell derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), ascorbic acid and cyclic AMP (cAMP). After 14 days of differentiation in BrainPhys™ Neuronal Medium, neurons express the synaptic marker Synapsin 1 and the mature neuronal marker MAP2, consistent with neurons cultured in a traditional basal medium under the same supplementation conditions (DMEM/F12) (Figure 6A-B). After 44 days of differentiation and maturation in BrainPhys™ Neuronal Medium, a large number of viable neurons with extensive branching make up a dense neurite network that can be observed under phase-contrast microscopy (Figure 7A). In comparison, traditional cultures show increased neuronal vacuolation and cellular debris after differentiation and maturation in a traditional culture medium. (Figure 7B). Marker analysis confirms that neurons cultured in BrainPhys™ Neuronal Medium express MAP2 and Synapsin 1 after 44 days in vitro (Figure 6C). Patch clamp analysis shows that neurons cultured in BrainPhys™ Neuronal Medium are functionally mature and show improved synaptic activity, compared to those cultured in a traditional basal medium (Figure 8). The frequency and amplitude of spontaneous excitatory (AMPA receptor-mediated) and inhibitory (GABA receptor-mediated) synaptic currents are increased in BrainPhys™ Neuronal Medium-matured cultures, compared to neurons matured in a traditional medium. hPSC-derived neurons have been successfully matured in BrainPhys™ Neuronal Medium for up to 70 days in vitro.

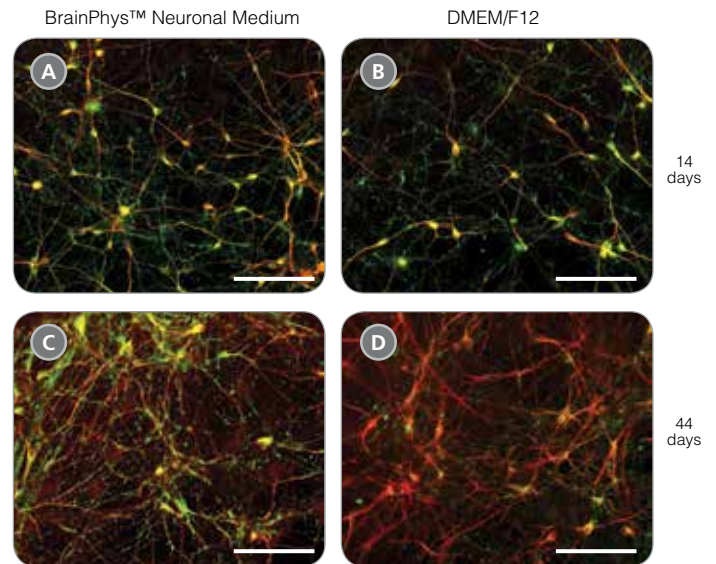


Figure 6. hPSC-Derived Neurons Generated in BrainPhys™ Neuronal Medium Express Markers of Neuronal Maturity After 14 and 44 Days of Differentiation

NPCs were generated from H9 cells using STEMdiff™ Neural Induction Medium in an embryoid body-based protocol. Next, NPCs were cultured in (A,C) BrainPhys™ Neuronal Medium, supplemented with 2% NeuroCult™ SM1 Supplement, 1% N2 Supplement-A, 20 ng/mL GDNF, 20 ng/mL BDNF, 1 mM db-cAMP and 200 nM ascorbic acid to initiate neuronal differentiation, or (B,D) DMEM/F12 under the same supplementation conditions. After 14 and 44 days of differentiation and maturation, neurons express the synaptic marker Synapsin 1 (green) and the mature neuronal marker MAP2 (red). In this example, neurons matured in BrainPhys™ Neuronal Medium show increased Synapsin 1 expression. Scale bar= 100 μm.

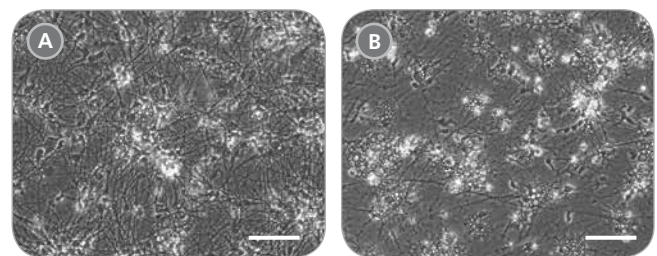


Figure 7. hPSC-Derived Neurons Generated in BrainPhys™ Neuronal Medium and NeuroCult™ SM1 and N2 Supplements are Healthy and Morphologically Normal

NPCs were generated from H9 cells using STEMdiff™ Neural Induction Medium in an embryoid body-based protocol. Next, NPCs were cultured for 44 DIV in (A) BrainPhys™ Neuronal Medium, supplemented with 2% NeuroCult™ SM1 Supplement, 1% N2 Supplement-A, 20 ng/mL GDNF, 20 ng/mL BDNF, 1 mM db-cAMP and 200 nM ascorbic acid to initiate neuronal differentiation, or (B) DMEM/F12 under the same supplementation conditions. Neuronal cultures differentiated from NPCs in BrainPhys™ Neuronal Medium display extensive neurite outgrowth and reduced cellular debris compared to cultures differentiated in DMEM/F12. Scale bar= 100 μm.

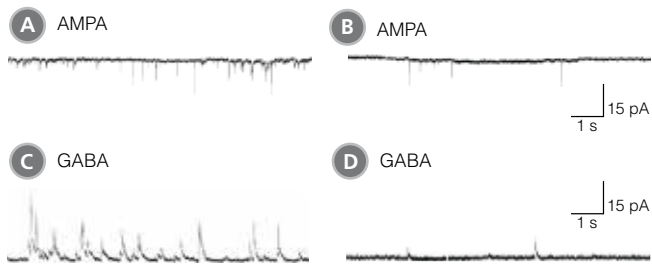


Figure 8. hPSC-Derived Neurons Matured in BrainPhys™ Neuronal Medium Show Improved Excitatory and Inhibitory Synaptic Activity

NPCs were generated from H9 cells using STEMdiff™ Neural Induction Medium in an embryoid body-based protocol. Next, NPCs were cultured for 44 DIV in (A,C) BrainPhys™ Neuronal Medium, supplemented with 2% NeuroCult™ SM1 Supplement, 1% N2 Supplement-A, 20 ng/mL GDNF, 20 ng/mL BDNF, 1 mM db-cAMP and 200 nM ascorbic acid to initiate neuronal differentiation, or (B,D) in DMEM/F12 under the same supplementation conditions. (A,C) Neurons matured in BrainPhys™ Neuronal Medium showed spontaneous excitatory (AMPA-mediated; A) and inhibitory (GABA-mediated; C) synaptic events. The frequency and amplitude of spontaneous synaptic events is consistently greater in neuronal cultures matured in BrainPhys™ Neuronal Medium, compared to neurons plated and matured in DMEM/F12 (B,D). Traces are representative.

Product Information

Neuronal Media and Supplements for Culture and Maturation

PRODUCT	SIZE	CATALOG #
BrainPhys™ Neuronal Medium	500 mL	05790
BrainPhys™ Neuronal Medium and SM 1 Kit *	1 Kit	05792
BrainPhys™ Neuronal Medium N2-A/SM 1 Kit *	1 Kit	05793
NeuroCult™ SM1 Neuronal Supplement (50X)	10 mL	05711
N2 Supplement-A (100X)	5 mL	07152
N2 Supplement-B (100X)	5 mL	07156

*Kit includes basal medium plus supplements

References

- Bardy C et al. (2015) Proc Natl Acad Sci 112 (20) E2725-E2734.
- Brewer GJ et al. (1993) J Neurosci Res. 35(5):567-76.
- Bottenstein JE (1985) Cell Culture in the Neurosciences: Current Topics in Neurobiology. New York: Plenum Press: 3-40.

Supplementary Reagents

Small Molecules for Neural Differentiation*

MOLECULE	PATHWAY/TARGET	CATALOG #
Purmorphamine	Hedgehog pathway activator Activates smoothened	72202
All-Trans Retinoic Acid	Activates RAR	72262
Valproic Acid	Epigenetic modifier Inhibits histone deacetylase (HDAC1)	72292
CHIR99021	WNT pathway activator Inhibits GSK3	72052
DAPT	Notch pathway inhibitor Inhibits γ -secretase	72082
SB431542	TGF- β pathway inhibitor Inhibits ALK5, ALK4, ALK7	72232
Forskolin	cAMP pathway activator Activates adenylyl cyclase	72112

*selected products only, see www.stemcell.com/smallmolecules for more

Antibodies for Neural Differentiation*

PRODUCT	HOST SPECIES	CATALOG #
Neuronal Class III beta-Tubulin Antibody, Clone TUJ1	Mouse	60052
Anti-Human Nestin Antibody, Clone 10C2	Mouse	60091
MAP2 Antibody, Clone AP20	Mouse	60049
GFAP Antibody	Rabbit	60128

*selected products only, see www.stemcell.com/antibodies for more

Cytokines for Neural Differentiation*

PRODUCT	SIZE	CATALOG #
BDNF, Human, Recombinant	5 μ g	02519
bFGF, Human, Recombinant	50 μ g	78003
EGF, Human, Recombinant	500 μ g	78006

*selected products only, see www.stemcell.com/cytokines for more

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