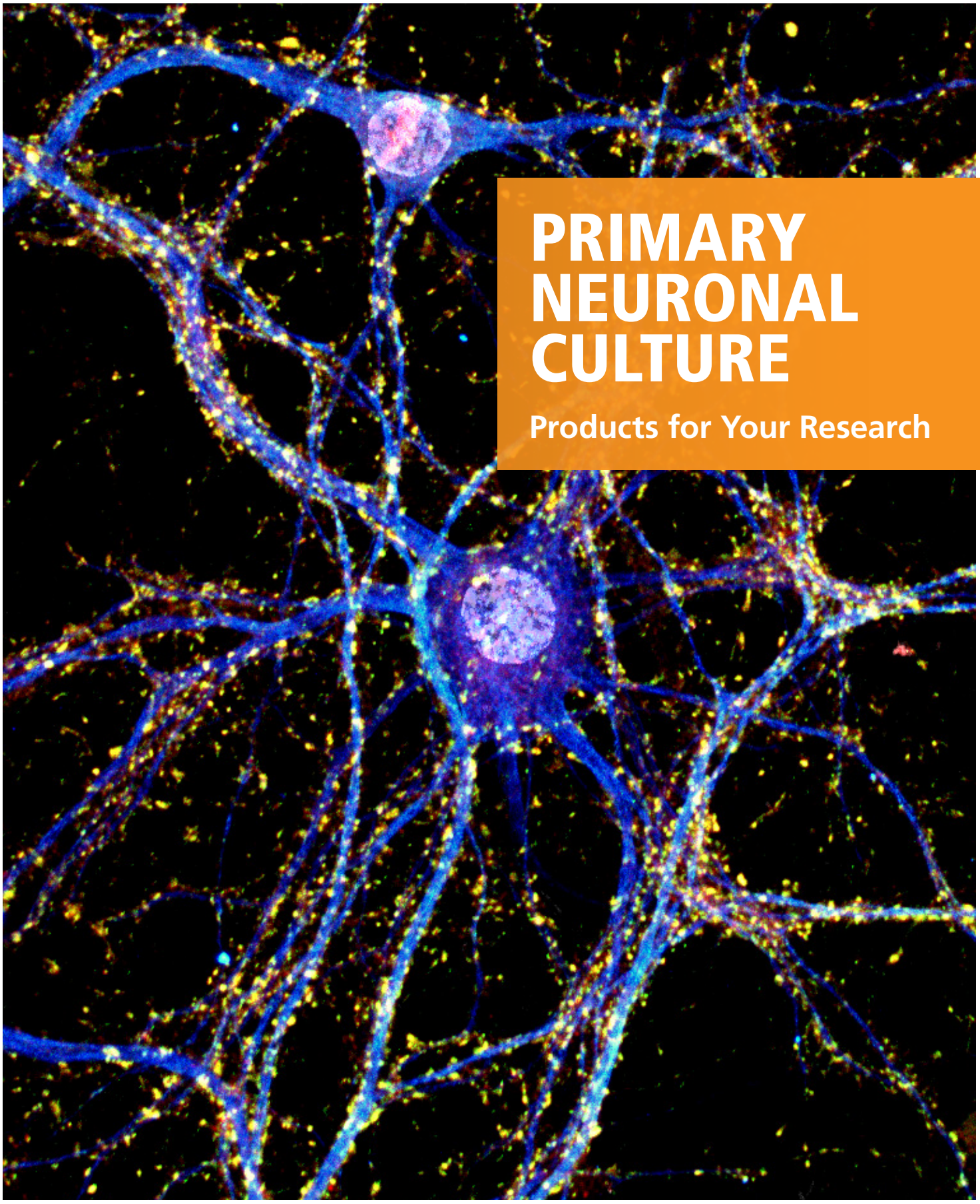

PRIMARY NEURONAL CULTURE

Products for Your Research

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TABLE OF CONTENTS

Primary Neuronal Culture

NeuroCult™ SM1 and BrainPhys™ Neuronal Medium

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 it is crucial to use high-quality media and supplements.[1-3] Variability in the quality of any of these raw materials, or in the associated manufacturing processes, results in inconsistent reagent quality, which negatively impacts sensitive neuronal cultures.[3,4]

NeuroCult™ SM (STEMCELL-Modified) Neuronal Supplements are based on Brewer's B27 supplement,[1] and optimized to more consistently support the culture of mature, functional neurons in both short- and long-term cultures.

NeuroCult™ SM Supplements may be combined with BrainPhys™ Neuronal Medium, a neuronal basal medium designed by Dr. Cedric Bardy in Dr. Fred H. Gage's laboratory to better support the in vitro neuronal function of both primary and human pluripotent stem cell (hPSC)-derived neurons.[5]

When culturing primary neurons, it is optimal to use a two-step protocol that takes into account different culture needs at different stages: an initial survival-optimized medium for plating and a function-optimized medium for subsequent maturation and long-term culture. The BrainPhys™ Primary Neuron Kit includes the media and supplement necessary for such a two-step protocol.

Educational Webinar

Dr. Cedric Bardy discusses why and how he created BrainPhys™.

Topics:

- Overview of the media used for neuronal cultures
- Electrophysiological measurements of human neural cultures
- The advantages of neuronal culture media adapted for neurophysiological activity

WEBINAR
The Road to Functional Human Neuronal Circuits in Vitro
www.stemcell.com/BrainPhysWebinar

STEMCELL Products For Every Step of Your Primary Neuronal Research

Tissue Dissociation	Cell Culture	Characterization
• Papain	• BrainPhys™ Neuronal Medium • BrainPhys™ Without Phenol Red • NeuroCult™ SM1 Neuronal Supplement • BrainPhys™ Primary Neuron Kit • BrainPhys™ Neuronal Medium and SM1 Kit • Cytokines • Small Molecules • Antibodies	• NeuroFluor™ NeuO • Cytokines • Antibodies

Increased Neuronal Viability
With the SM1 Culture System

NeuroCult™ SM1 Neuronal Supplement (NeuroCult™ SM1) is a serum-free supplement for the culture of rodent primary neurons and the differentiation and maturation of hPSCs. NeuroCult™ SM1 is designed based on the published B27 formulation[1,2] but optimized to more consistently support the culture of mature, functional neurons, whilst minimizing glial cell contamination (<1% GFAP). NeuroCult™ SM1 is available separately, as part of the BrainPhys™ Neuronal Medium and SM1 Kit, or as part of the BrainPhys™ Primary Neuron Kit.

For optimal neuronal survival and function, primary neurons are first plated in a survival-optimized medium (NeuroCult™ Neuronal Plating Medium with NeuroCult™ SM1) and then transitioned to a function-optimized medium (BrainPhys™ Neuronal Medium with NeuroCult™ SM1) (Figure 1). These basal media (NeuroCult™ Neuronal Plating Medium and BrainPhys™ Neuronal Medium) and supplement (NeuroCult™ SM1) are the components of the BrainPhys™ Primary Neuron Kit.

Neurons cultured with the SM1 Culture System (i.e. BrainPhys™ Primary Neuron Kit) are morphologically mature, with neurites that have developed into elaborate networks of processes (Figure 2), and show punctate expression of pre- and post-synaptic markers at 21 days in vitro (DIV) (Synapsin, MAP2 and PSD-95; Figure 3).

The SM1 Culture System was compared to a Competitor Culture System (Neurobasal® supplemented with B-27™). A significantly higher number of class III ß-tubulin-immunoreactive neurons was observed when neurons were cultured in the SM1 Culture System compared to the Competitor Culture system, indicating significantly improved cell survival (Figure 4A). Further comparison of NeuroCult™ SM1 to B27-like competitors shows that when a neuronal basal medium (Neurobasal®) is used throughout the culture period, the number of neurons is comparable in the NeuroCult™ SM1-supplemented cultures (Figure 4B). This ability to support neurons with high viability in culture for 21 DIV is highly consistent among different lots of NeuroCult™ SM1. Furthermore, we have previously shown that neurons cultured in NeuroCult™ SM1-supplemented media show significantly greater total neurite outgrowth, greater number of neurite branch points, and normal electrophysiological profiles (data not shown).

Now Available

NeuroCult™ SM1 Without Vitamin A

NeuroCult™ SM1 Without Antioxidants

NeuroCult™ SM1 Without Insulin

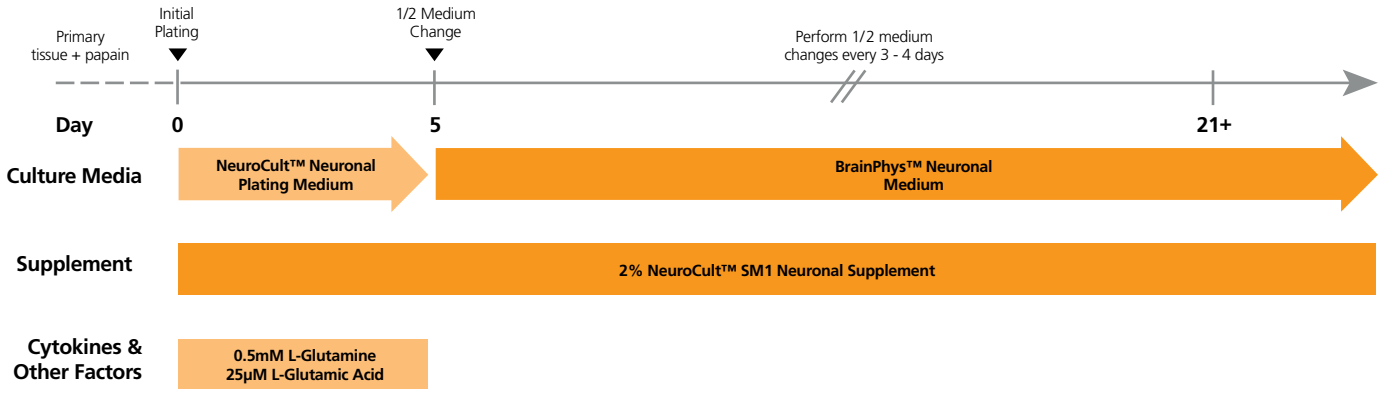

Figure 1. Protocol for Plating and Culturing Primary Neurons with the SM1 Culture System

Primary rodent tissue dissociated in papain was plated in NeuroCult™ Neuronal Plating Medium, supplemented with NeuroCult™ SM1 Neuronal Supplement, L-Glutamine, and L-Glutamic Acid. On day 5, primary neurons were transitioned to BrainPhys™ Neuronal Medium, supplemented with NeuroCult™ SM1 Neuronal Supplement, by performing half-medium changes every 3 - 4 days.

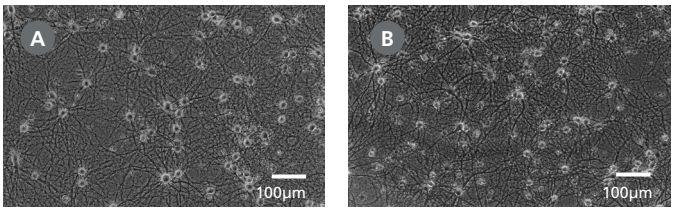

Figure 2. The SM1 Culture System Supports Long-Term Culture of Rodent Neurons

Primary E18 rat cortical neurons were cultured in the SM1 Culture System. A large number of viable neurons are visible after (A) 21 and (B) 35 days, as demonstrated by their bright neuronal cell bodies, and extensive neurite outgrowth and branching. Neurons are evenly distributed over the culture surface with minimal cell clumping.

Why Use NeuroCult™ SM1?

SPECIALIZED. NeuroCult™ SM1 is formulated to support improved cell survival in long-term primary neuronal culture.

OPTIMIZED. Cultures feature increased neurite outgrowth and branching in short- and long-term cultures.

RELIABLE AND CONSISTENT. Product undergoes rigorous quality control testing to ensure consistent results.

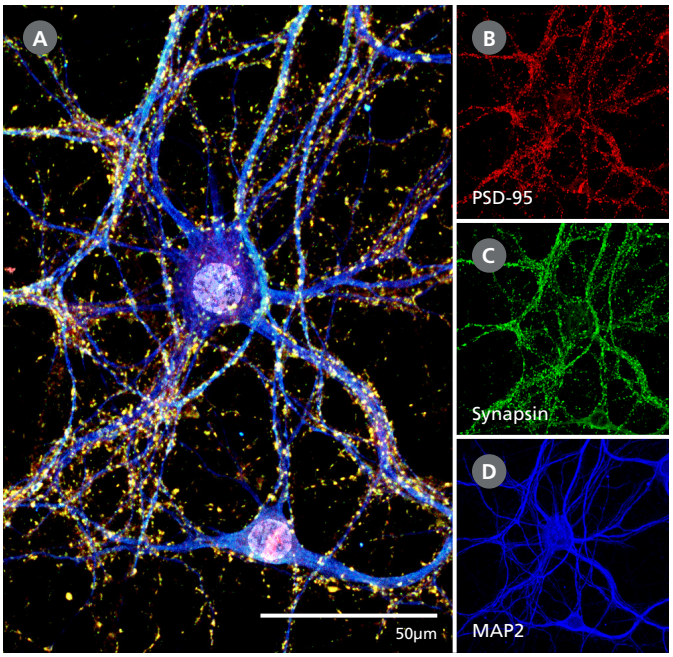

Figure 3. Pre- and Post-Synaptic Markers are Expressed in Rodent Neurons Cultured in the SM1 Culture System

Primary E18 rat cortical neurons were cultured in the SM1 Culture System. At 21 DIV, neurons are phenotypically mature, as indicated by the presence of an extensive dendritic arbor, and appropriate expression and localization of pre-synaptic synapsin (A,C; green) and post-synaptic PSD-95 (A,B; red) markers. Synapsin is concentrated in discrete puncta distributed along the somata and dendritic processes, as defined by the dendritic marker MAP2 (A,D; blue).

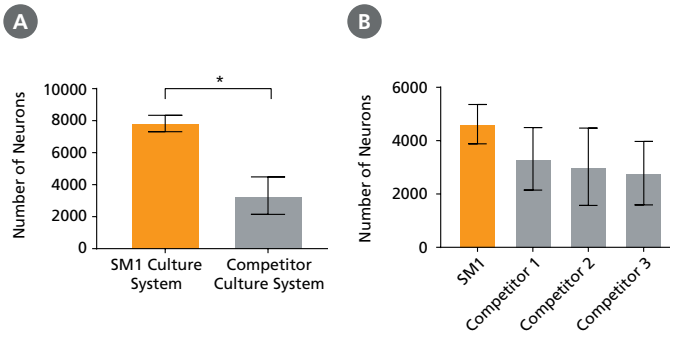

Figure 4. The SM1 Culture System Supports Increased Cell Survival

(A) Primary E18 rat cortical neurons were cultured in the SM1 Culture System or a Competitor Culture System (Neurobasal® supplemented with B-27™) for 21 days. Neurons cultured in the SM1 Culture System have a significantly higher number of viable cells compared to the competitor culture system (n = 4; mean ± 95% CI; *$p < 0.05$). (B) Primary E18 rat cortical neurons were cultured in Neurobasal® supplemented with NeuroCult™ SM1 Neuronal Supplement (SM1) or competitor B27-like supplements (Competitor 1,2,3) for 21 days. Cultures supplemented with NeuroCult™ SM1 Neuronal Supplement have an equal number of neurons compared to competitor-supplemented cultures. Bars represent standard error of mean.

Increased Function and Maturation

With BrainPhys™ Neuronal Medium

BrainPhys™ Neuronal Medium is a serum-free neuronal basal medium for the culture of rodent primary neurons and the neuronal differentiation and maturation of hPSCs. Based on the formulation invented by Dr. Cedric Bardy and Dr. Fred H. Gage,[5] BrainPhys™ Neuronal Medium is more representative of the brain's extracellular environment and increases the proportion of synaptically active neurons. In his original paper,[5] Dr. Bardy found that traditional neuronal culture media (e.g. 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).[5]

BrainPhys™ Neuronal Medium is available separately, in a phenol red-free format, as part of the BrainPhys™ Neuronal Medium and SM1 Kit, or as part of the BrainPhys™ Primary Neuron Kit. Alternative kits are available for the differentiation and maturation of hPSCs.

As previously shown, neurons cultured in the SM1 Culture System show large numbers of viable neurons that are phenotypically mature (Figures 2 - 4).

Neurons matured in BrainPhys™ Neuronal Medium are functionally mature and show improved synaptic activity compared to those cultured in a competitor neuronal medium (Neurobasal®; Figure 5). 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, when assessed with a microelectrode array 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 competitor neuronal medium (Figure 6).

Applications of BrainPhys™ Neuronal Medium

- Culture of primary mouse or rat neurons.
- Neuronal differentiation and maturation of hPSC-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 neurons.[5]

Table 1. Properties of Culture Media[5]

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	Not tested	✓
Excitatory synaptic activity	Blocked	Low	✓
Inhibitory synaptic activity	Blocked	Blocked	✓

Check-mark denotes physiological conditions and supported activities according to Bardy et al.[5]

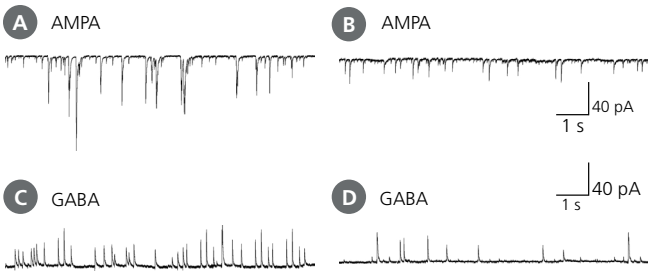

Figure 5. Rodent Neuronal Cultures Matured in BrainPhys™ Neuronal Medium Show Improved Excitatory and Inhibitory Synaptic Activity

(A,C) Primary rat E18 cortical neurons were plated in NeuroCult™ Neuronal Basal Medium (product superseded with NeuroCult™ Neuronal Plating Medium which is a part of the BrainPhys™ Primary Neuron Kit), 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 competitor neuronal medium (Neurobasal®), 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 competitor neuronal medium (B,D). Traces are representative.

Why Use BrainPhys™ Neuronal Medium?

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 primary- and hPSC-derived neurons.

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

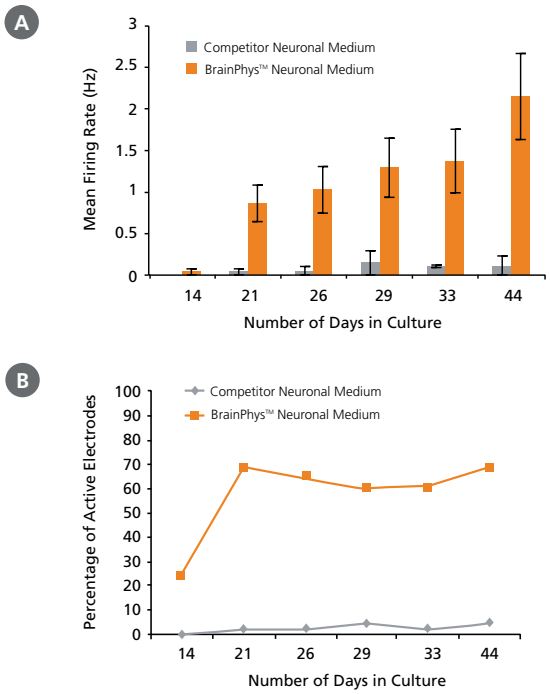

Figure 6. Primary Neuronal Cultures Matured in BrainPhys™ Neuronal Medium Show Improved Electrical Activity in Microelectrode-Array Systems

Primary rat E18 cortical neurons were plated in a competitor neuronal medium (Neurobasal®) supplemented with NeuroCult™ SM1 Neuronal Supplement. After 5 DIV, half of the cultures were transitioned to BrainPhys™ Neuronal Medium, supplemented with NeuroCult™ SM1 Neuronal Supplement, by performing half-medium changes every 3 - 4 days. The other half of the cultures were maintained in the competitor neuronal medium 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 increases over time, whereas the mean firing rate of neurons in the competitor neuronal medium condition remains low (n = 1; mean ± SEM, 128 electrodes). (B) The percentage of active electrodes (>0.005 Hz) of neurons matured in BrainPhys™ Neuronal Medium increases from 24% on day 14 to 69% on day 21, and then remains stable at 60 – 70% from days 21 – 44. In contrast, < 5% of electrodes was active in the competitor neuronal medium condition over the same 6-week period.

BRAINPHYS™ PUBLICATIONS

How is BrainPhys™ Being Used in Research?
www.stemcell.com/BrainPhysReferences

Characterization of Primary Neurons

With NeuroFluor™ NeuO

NeuroFluor™ NeuO is a membrane-permeable fluorescent probe that selectively labels primary and pluripotent stem cell-derived neurons in live cultures.[6] Cells labeled with NeuroFluor™ NeuO can be visualized using fluorescent imaging. Labeling with this probe is non-permanent; it can be washed off, providing unlabeled, viable cells for downstream applications. Fluorescent properties: excitation 468 nm, emission 557 nm.

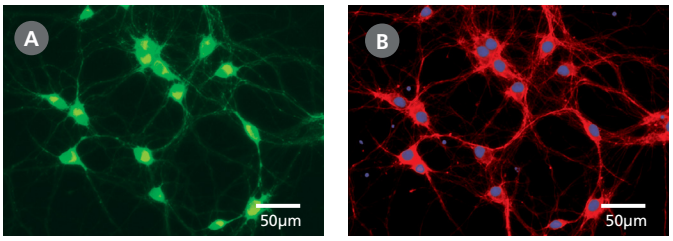

Figure 7. NeuroFluor™ NeuO Selectively Labels Primary and hPSC-Derived Neurons

(A) Neurons derived from primary rat cortical tissues were cultured in BrainPhys™ Neuronal Medium with NeuroCult™ SM1 Neuronal Supplement. After 8 days of culture, primary neurons were labeled with NeuroFluor™ NeuO (green). (B) The same culture was later fixed and immunostained for class III β-tubulin (red). Nuclei are counterstained with DAPI. The images show that NeuroFluor™ NeuO specifically labels class III β-tubulin-positive neurons.

Why Use NeuroFluor™ NeuO?

NO FIXATION. Enables selective labeling of primary- and hPSC-derived neurons without fixation.

NON-PERMANENT. Non-toxic and can be washed off.

VERSATILE. Can be used to confirm neuronal differentiation of human pluripotent stem cell-derived NPCs.

FUNCTIONAL. Can be used to label neurons in live culture.

CONVENIENT. Simple and rapid labeling protocol.

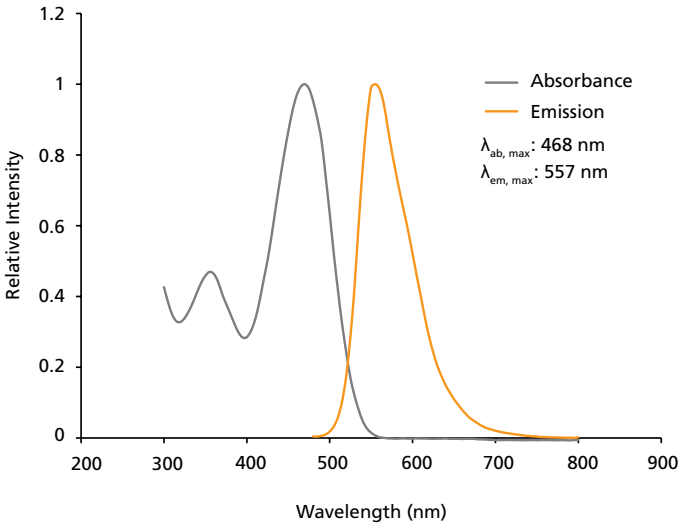

Figure 8. Fluorescent Properties of NeuroFluor™ NeuO

Excitation 468 nm, emission 557 nm.

Applications of NeuroFluor™ NeuO

- Label live hPSC-derived neurons in culture without fixation.
- Label tissue sections of mouse brain through an intravenous injection of NeuroFluor™ NeuO.
- Locate neuronal cell bodies for electrophysiology experiments.
- Isolate live neurons from rodent brain tissues using fluorescence-activated cell sorting (FACS).

Product Information

PRODUCT	SIZE	CATALOG #
BrainPhys™ Basal Media		
BrainPhys™ Neuronal Medium	500 mL	05790
BrainPhys™ Without Phenol Red	500 mL	05791
NeuroCult™ SM Supplements		
NeuroCult™ SM1 Neuronal Supplement (50X)	10 mL	05711
NeuroCult™ SM1 Without Vitamin A (50X)	10 mL	05731
NeuroCult™ SM1 Without Antioxidants (50X)	10 mL	05732
NeuroCult™ SM1 Without Insulin (50X)	10 mL	05733
Complete Kits		
BrainPhys™ Neuronal Medium and SM1 Kit	1 Kit*	05792
BrainPhys™ Primary Neuron Kit	1 Kit**	05794
Other Products		
BrainPhysNeuroFluor™ NeuO	0.1 mL	01801

*Kit includes BrainPhys™ Neuronal Medium and NeuroCult™ SM1 Neuronal Supplement.
**Kit includes BrainPhys™ Neuronal Medium, NeuroCult™ SM1 Neuronal Supplement, and NeuroCult™ Neuronal Plating Medium.

Supplementary Reagents

Cytokines

Activate, expand, and differentiate cells with cytokines and growth factors. These high-quality reagents support neuronal cultures and ensure reproducibility across a variety of assays. Choose from a large selection of human, mouse, and rat cytokines to incorporate into your research workflow. For a complete listing of cytokines available, including animal-component free versions, please visit **www.stemcell.com/cytokines**.

CYTOKINE	CATALOG #
BDNF	78005
bFGF	78133
CNTF	78010
EGF	78006
EGFR	78171
FGF-17	78179
FGF-5	78182
FGF-8A	78128
FGF-acidic	78187
G-CSF	78012

CYTOKINE	CATALOG #
GDNF	78058
Heregulin-β1	78071
HGF	78019
IGF-II	78023
IL-4	78045
IL-6	78050
IL-10	78024
IL-11	78025
LIF	78055
M-CSF	78057

CYTOKINE	CATALOG #
NGF-β	78092
Noggin	78060
NOV	78198
NT-3	78093
Oncostatin M	78094
PDGF (variants)	7809
Persephin	78200
Prolactin	78098
TGF-β1	78067
TNF-α	78068

Antibodies

Analyze cells with antibodies that are verified to work with STEMCELL's cell culture reagents in select applications. Choose from a range of antibodies specifically selected for neuronal research to ensure consistent results in your downstream applications, including immunofluorescence and immunocytochemistry. For a complete listing of available antibodies and conjugates, visit **www.stemcell.com/antibodies**.

TARGET ANTIGEN	CLONE	ISOTYPE	APPLICATIONS	SIZE	CATALOG #
Neuronal Markers					
β-Tubulin III	TUJ1	Mouse IgG_{2a}	ICC, IF	250 µL	60052
β-Tubulin III	2G10-TB3	Mouse IgG_{2a}	ICC, IF, WB	100 µg	60092
				25 µg	60092.1
β-Tubulin III	AA10	Mouse IgG_{2a}	ICC, IF, WB	100 µg	60100
				25 µg	60100.1
Tyrosine Hydroxylase	TH-2	Mouse IgG_1	ICC	200 µL	60058
Glial Markers					
Glial Fibrillary Acidic Protein (GFAP)	-	Rabbit Polyclonal	IHC, WB	200 µL	60128
	2E1.E9	Mouse IgG_{2b}	FC, ICC, IF, WB	100 µg	60048
				25 µg	60048.1
Oligodendrocyte Marker O4	81	Mouse IgM	ICC	50 µg	60053
Central/Peripheral Nervous System Marker					
NGF Receptor/p75NTR (CD271)	192-IgG (MC192)	Mouse IgG_1	IHC	100 µg	60101
Neural Stem Cell Markers					
Nestin	Rat401	Mouse IgG_1	ICC	100 µg	60051
	10C2	Mouse IgG_1	FC, ICC, IF, WB	100 µg	60091
				25 µg	60091.1

*Abbreviations: FC: Flow Cytometry; ICC: Immunocytochemistry; IF: Immunofluorescence Microscopy; IHC: Immunohistochemistry; WB: Western Blotting

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. Brewer GJ and Cotman CW. (1989) Survival and growth of hippocampal neurons in defined medium at low density: advantages of a sandwich culture technique or low oxygen. Brain Res. 494(1):65-74.
3. Chen Y et al. (2008) NS21: re-defined and modified supplement B27 for neuronal cultures. J Neurosci Methods 171(2):239-47.
4. Cressey D. (2009) Neuroscientists claim growing pains. Nature 459: 19.
5. 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.
6. Er JC et al. (2017) NeuO: a flurorescent chemical probe for live neuron labeling. Angew Chem Int Ed 54(8) 2442-6.

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