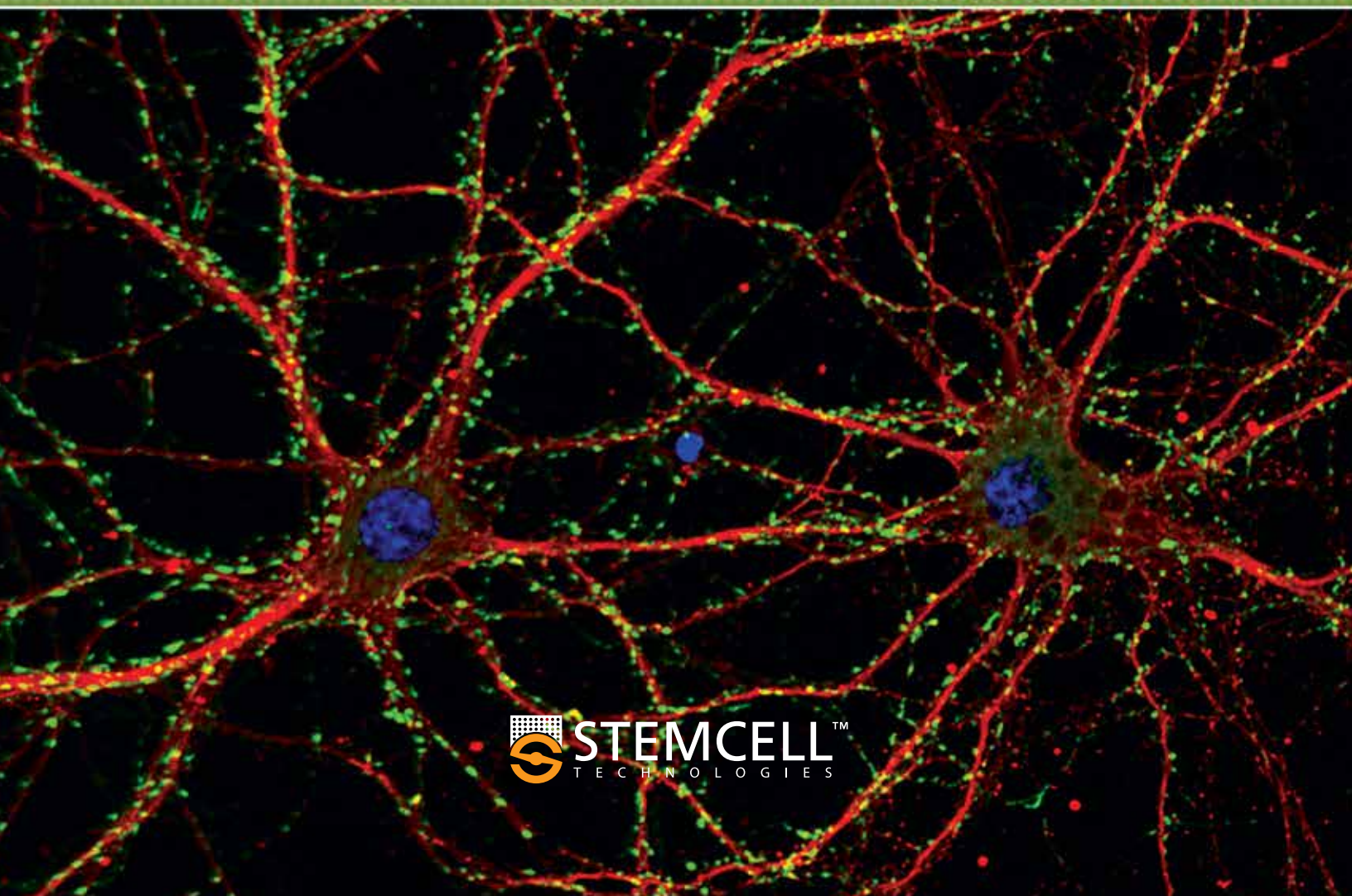
Primary Neuronal Culture

BrainPhys™ and NeuroCult™ Neuronal Media and Supplements



STEMCELL™ TECHNOLOGIES

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Front cover. Primary neurons cultured in NeuroCult™ SM1-supplemented NeuroCult™ Neuronal Basal Medium for 21 days.

Superior Primary Neuronal Culture

With Standardized NeuroCult™ Reagents 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, using high-quality media and supplements is crucial, because they contain numerous complex components.[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 NeuroCult™ Neuronal Basal Medium or with BrainPhys™ Neuronal Medium. NeuroCult™ Neuronal Basal Medium is based on Brewer's Neurobasal medium,[1] which was designed for optimal survival of neurons, and is the ideal choice for generating traditional primary neuronal cultures. BrainPhys™ Neuronal Medium is a new 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]

Optimal Neuronal Function and Survival

The BrainPhys™ Neuronal Medium and SM1 Kit includes the best of both worlds: the consistent NeuroCult™ SM1 and the functional BrainPhys™ Neuronal Medium.

STEMCELL Products For Every Step of Your Neuronal Research

Tissue Dissociation	Cell Culture	Characterization
• Trypsin-EDTA	• BrainPhys™ Neuronal Medium • BrainPhys™ Neuronal Medium and SM1 Kit • NeuroCult™ SM1 Neuronal Supplement • NeuroCult™ SM1 Neuronal Culture Kit • NeuroCult™ SM2 Neuronal Supplement • NeuroCult™ Neuronal Basal Medium • Cytokines • Antibodies	• Antibodies

Traditional Neuronal Culture

Increases Survival of Primary Neurons in Long-Term Culture

NeuroCult™ SM1 Neuronal Supplement 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 NeuroCult™ SM1 Neuronal Culture Kit (in which it is complemented by NeuroCult™ Neuronal Basal Medium), or as part of the BrainPhys™ Neuronal Medium and SM1 Kit (see page 7).

Primary neurons cultured for 7 days in vitro (DIV) in NeuroCult™ SM1-supplemented media have normal morphology (Figure 1A) and exhibit less cell body and neurite clumping, compared to neurons cultured in an alternative (Neurobasal) serum-free supplemented media (ASSM; data not shown). At 21 DIV, neuronal viability remains high, and neurites have developed into elaborate networks of processes, indicative of healthy, mature cultures (Figure 1B).

A significantly higher number of class III ß-tubulin-immunoreactive neurons can be observed when neurons are cultured in NeuroCult™ SM1-supplemented media for 21 DIV compared to ASSM, indicating significantly improved cell survival (Figure 2B). This ability to support neurons with high viability in culture for 21 DIV is highly consistent among different lots of NeuroCult™ SM1. Furthermore, neurons cultured for both 7 and 21 DIV in NeuroCult™ SM1-supplemented media show significantly greater total neurite outgrowth (Figure 3) and number of neurite branch points (data not shown), compared to ASSM. Neurite outgrowth and branching serve as indicators of the extent of neuronal maturation in culture.

Neurons cultured in NeuroCult™ SM1-supplemented media are morphologically mature and show punctate expression of pre- and post-synaptic markers at 21 DIV (synapsin and PSD-95, respectively; Figure 4). The functional maturity of neurons cultured in this system is demonstrated by the fact that the neurons exhibit normal electrophysiological profiles (Figure 5).

Now available

NeuroCult™ SM1 Without Vitamin A

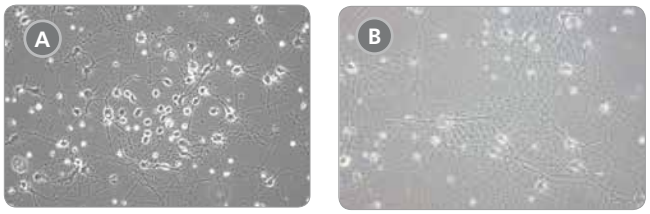


Figure 1. Morphology of Neuronal Cultures Maintained in NeuroCult™ SM1-Supplemented Media

Primary rat E18 rat cortical neurons were cultured for (A) 7 and (B) 21 DIV in NeuroCult™ SM1-supplemented media. (A) Large numbers of viable neurons are visible, with minimal cell clumping and extensive neurite outgrowth and branching. (B) Large numbers of viable neurons with developed dendritic arbors remain in culture. Magnification: 20x.

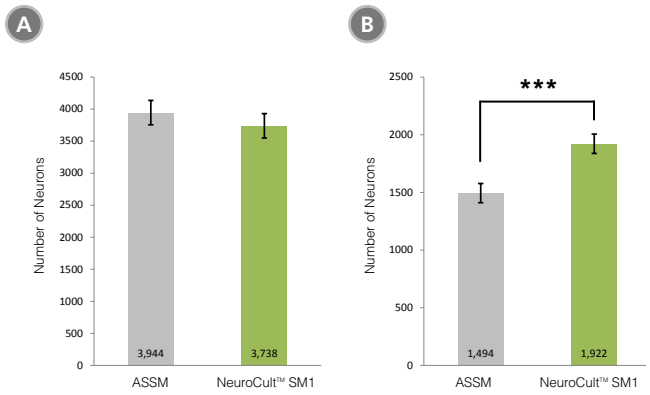


Figure 2. Number of Neurons Cultured in NeuroCult™ SM1-Supplemented Media and ASSM

Primary rat E18 rat cortical neurons were cultured for (A) 7 and (B) 21 DIV in NeuroCult™ SM1-supplemented media (NeuroCult™ SM1) and ASSM. (A) At 7 days, numbers of neurons are comparable (n = 25; mean ± 95% CI; $p > 0.05$) but after (B) 21 days, NeuroCult™ SM1 cultures have significantly higher numbers of neurons compared to ASSM (n = 25; mean ± 95% CI; ***$p < 0.001$).

Advantages of 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. Product undergoes rigorous quality control testing.

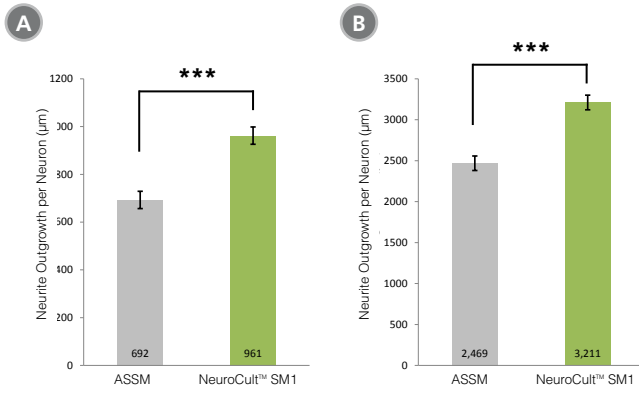


Figure 3. Neurite Outgrowth of Neurons Cultured in NeuroCult™ SM1-Supplemented Media and ASSM

Primary rat E18 rat cortical neurons were cultured for (A) 7 and (B) 21 DIV in NeuroCult™ SM1-supplemented media (NeuroCult™ SM1) and ASSM. Significantly greater neurite outgrowth is observed for cells cultured in NeuroCult™ SM1, compared to ASSM (n = 240 independent measures; mean ± 95% CI; ***p < 0.001).

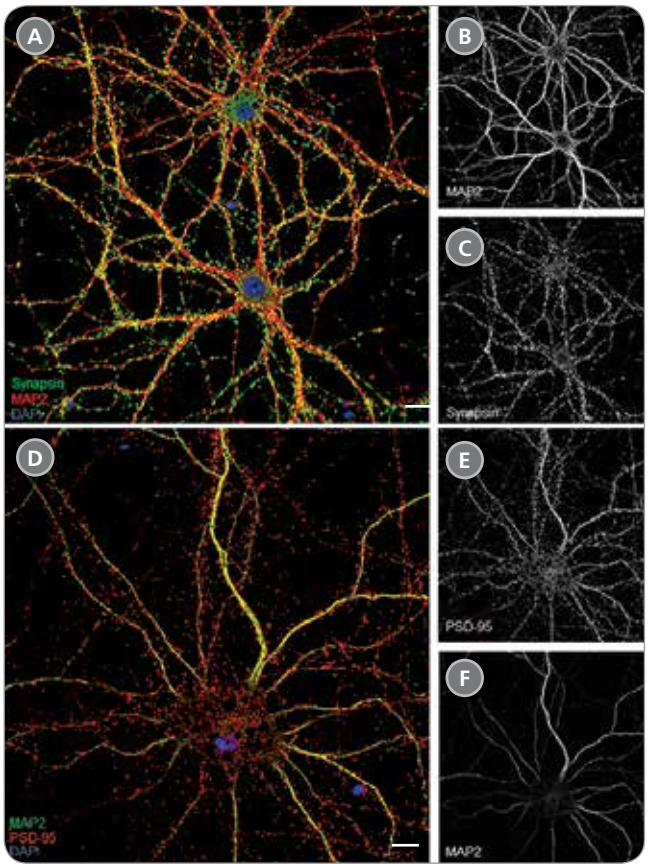
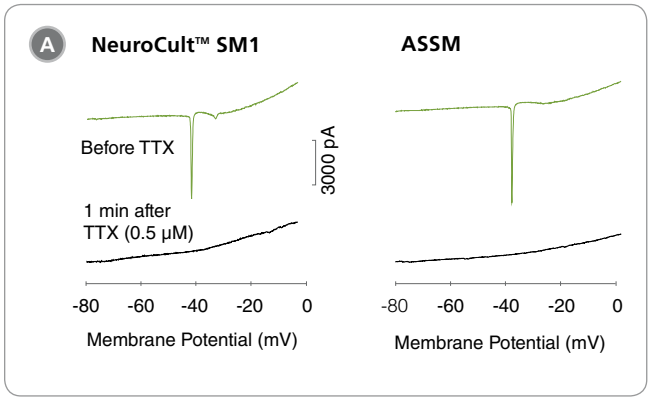


Figure 4. Expression of Pre- and Post-Synaptic Markers in Neurons Cultured in NeuroCult™ SM1-Supplemented Media

Neurons cultured in NeuroCult™ SM1-supplemented for 21 DIV are phenotypically mature, as indicated by the presence of an extensive dendritic arbor, and appropriate expression and localization of pre-synaptic (synapsin) and post-synaptic (PSD-95) markers. (A-C) Synapsin (green) immunolabeling is concentrated in discrete puncta distributed along the somata and dendritic processes, as defined by MAP2 (red) staining. (D-F) Dendritic immunolabeling observed for MAP2 (green) and punctate staining for the postsynaptic marker PSD-95 (red). Nuclei were counter-stained with DAPI. Scale bar= 10 µm.

Substrate-Independent Culture

Eliminate variability due to substrate with NeuroCult™ SM2 Neuronal Supplement



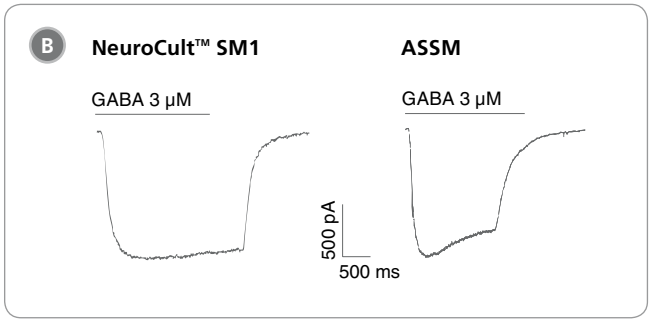


Figure 5. Electrophysiological Profile of Neurons Cultured in NeuroCult™ SM1-Supplemented Media

E14 mouse cortical neurons were cultured for 14 days on poly-D-lysine/laminin-coated plates in NeuroCult™ SM1-supplemented media (NeuroCult™ SM1). Neurons are functionally active, produce action potentials that are reversibly blocked by application of tetrodotoxin (TTX) and display typical ligand-induced $GABA_A$ receptor current profiles. (A) When measured in whole-cell configuration, voltage-gated sodium currents for these neurons were comparable to those of neurons cultured in ASSM. Sodium currents were recorded by a ramp test (depolarizing cell) from -80 to 0 mV in 200 ms. Currents were blocked by application of 0.5 µM tetrodotoxin (TTX). (B) Ligand-induced $GABA_A$ receptor currents for neurons cultured in NeuroCult™ SM1 were normal and comparable to those of neurons cultured in ASSM. Currents were evoked by direct fast application of 3 µM GABA to the test cell for 2 s.

PRODUCT NAME	UNIT SIZE	CATALOG #
NeuroCult™ SM1 Neuronal Supplement (50X)	10 mL	05711
NeuroCult™ SM1 Neuronal Culture Kit	500 mL*	05712
NeuroCult™ Neuronal Basal Medium	100 mL	05710
NeuroCult™ SM2 Neuronal Supplement (50X)	2 mL	05721

*Kit contains 1 unit of NeuroCult™ SM1 Neuronal Supplement and 5 units of NeuroCult™ Neuronal Basal Medium (100 mL).

TECHNICAL BULLETIN

NeuroCult™ SM1 for Long-Term Primary Neuronal Culture

www.stemcell.com/sm1longtermtb

Neurophysiologically Active Neuronal Culture

BrainPhys™ Neuronal Medium

Neurons cultured in BrainPhys™ Neuronal Medium show large numbers of viable neurons, with minimal cell clumping and extensive neurite outgrowth and branching (Figure 6A,C); the cells are phenotypically mature, as indicated by the highly branched morphology and appropriate expression of MAP2 and Synapsin 1 (Figure 7). Neuronal morphology is consistent with neurons cultured in an alternative neuronal medium (Neurobasal Medium; Figure 6B,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 an alternative neuronal medium is used as the initial plating medium (Figure 8).

When cultured in BrainPhys™ Neuronal Medium, neurons are functionally mature and show improved synaptic activity compared to those cultured in an alternative neuronal medium (Figure 9). 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 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 an alternative neuronal medium (Figure 10).

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 DIV.

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 neurons.[5]

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 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]

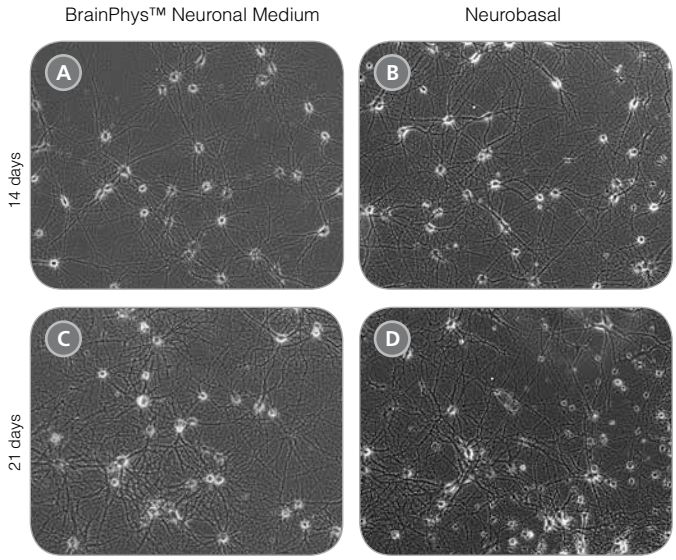


Figure 6. Rodent Neurons Matured in BrainPhys™ Neuronal Medium are Healthy and Morphologically Mature

(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 (A) 14 or (C) 21 DIV. (B,D) Primary rat E18 cortical neurons were plated and matured in an alternative neuronal medium (Neurobasal Medium), supplemented with NeuroCult™ SM1 Neuronal Supplement for (B) 14 or (D) 21 DIV. Neuronal morphology of BrainPhys™ Neuronal Medium-matured neurons is consistent with neurons plated and matured in an alternative neuronal medium.

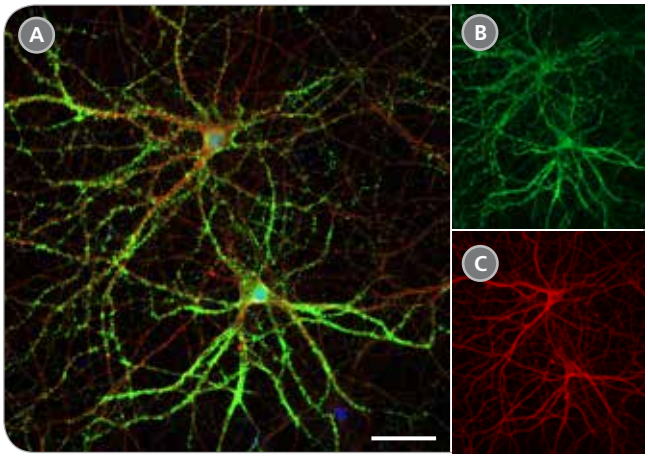


Figure 7. Expression of Pre-Synaptic Markers in Rodent Neurons Matured in BrainPhys™ Neuronal Medium

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 µm.

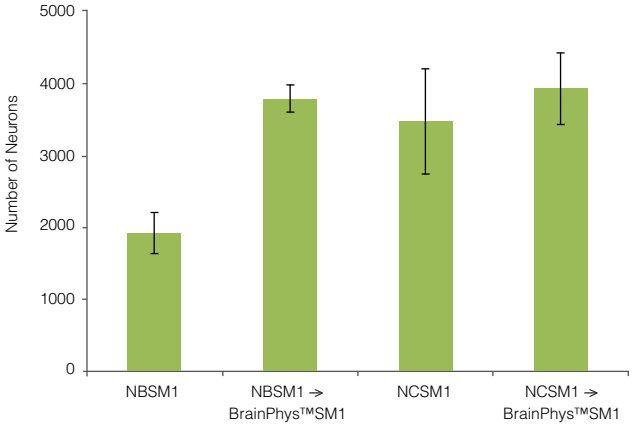


Figure 8. Primary Neuronal Cultures Matured in BrainPhys™ Neuronal Medium Have Greater Numbers of Neurons

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 = 4, mean ± SEM).

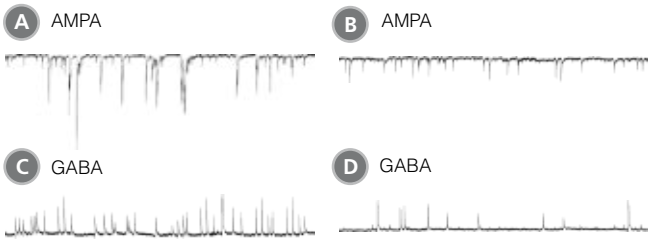


Figure 9. Rodent Neuronal Cultures Matured in BrainPhys™ Neuronal Medium Show Improved Excitatory and Inhibitory Synaptic Activity

(A,C) Primary rat E18 cortical neurons were plated in NeuroCult™ Neuronal Basal Medium, 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 an alternative 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 an alternative neuronal medium (B,D). Traces are representative.

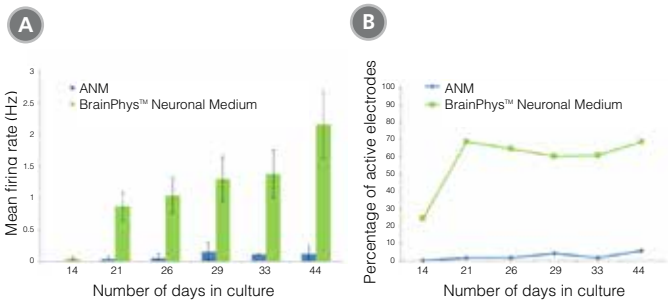


Figure 10. Primary Neuronal Cultures Matured in BrainPhys™ Neuronal Medium Show Improved Electrical Activity in Microelectrode-Array Systems

Primary rat E18 cortical neurons were plated in an alternative neuronal medium (ANM; 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 ANM 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 ANM condition remained low (n=1; mean ± 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 ANM condition over the same 6-week period.

PRODUCT NAME	UNIT SIZE	CATALOG #
BrainPhys™ Neuronal Medium	500 mL	05790
BrainPhys™ Neuronal Medium and SM1 Kit	1 Kit*	05792
NeuroCult™ Neuronal Basal Medium	100 mL	05710

*Kit includes basal medium plus supplements.

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www.stemcell.com/BrainPhysSample

Product Information

PRODUCT NAME	UNIT SIZE	CATALOG #
Basal Media		
BrainPhys™ Neuronal Medium	500 mL	05790
NeuroCult™ Neuronal Basal Medium	100 mL	05710
Supplements		
NeuroCult™ SM1 Neuronal Supplement (50X)	10 mL	05711
NeuroCult™ SM2 Neuronal Supplement (50X)	2 mL	05721
Complete Kits		
BrainPhys™ Neuronal Medium and SM1 Kit	1 Kit*	05792
NeuroCult™ SM1 Neuronal Culture Kit	500 mL**	05712
NeuroCult™SM2 Neuronal Culture Kit	100 mL	05722

*Kit includes basal medium plus supplements.
**Kit contains 1 unit of NeuroCult™ SM1 Neuronal Supplement and 5 units of NeuroCult™ Neuronal Basal Medium (100 mL).

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, 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, Stevens B, Chang J, Milbrandt J, Barres BA, Hell JW. NS21: re-defined and modified supplement B27 for neuronal cultures. J Neurosci Methods 171(2):239-47, 2008
4. Cressey D. Neuroscientists claim growing pains. Nature 459: 19, 2009
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.

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 cytokines and growth factors to incorporate into your research workflow. To view the full list of cytokines for neuronal research visit www.stemcell.com/cytokines.

CYTOKINE	QUANTITY	CATALOG #
Human Recombinant BDNF	5 µg	02519
Human Recombinant bFGF	10 µg	78003.1
	50 µg	78003
	1000 µg	78003.2
Human Recombinant EGF	100 µg	78006.1
	500 µg	78006
	1000 µg	78006.2
Human Recombinant EPO	500 U	02625
Human Recombinant IL-6	20 µg	78050.1
	100 µg	78050
	1000 µg	78050.2
Human Recombinant IL-11	10 µg	78025.1
	100 µg	78025
Human Recombinant LIF*	10 µg	78055.1
	50 µg	78055
	1000 µg	78055.2
Human Recombinant NT-4	5 µg	02509
Human Recombinant TGF-ß1	2 µg	02647
	10 µg	02847

Antibodies

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

TARGET ANTIGEN	CLONE	ISOTYPE	APPLICATIONS	QUANTITY	CATALOG #
Neuron Markers					
Microtubule Associated Protein 2 (MAP2)	AP20	Mouse IgG_1	ICC	100 µg	60049
				25 µg	60049.1
ß-Tubulin III	TUJ1	Mouse IgG_{2a}	ICC	250 µL	60052
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					
NGF Receptor/p75NTR (CD271)	MLR-2	Mouse IgG_{2a}	FC	100 µg	60102
	MLR-2	Mouse IgG_{2a}; FITC conjugated	FC	100 µg	60102FI
	192-IgG (MC192)	Mouse IgG_1	IHC	100 µg	60101
Neural Stem Cell Markers					
Nestin	Rat401	Mouse IgG_1	ICC	100 µg	60051
Sox-2	Poly6519	Rabbit Polyclonal	FC, ICC, IF, WB	200 µL	60055
				50 µL	60055.1

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

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