

# NEURAL SUPPLEMENTS

## For Consistent, High-Quality Cultures

The supplements required for consistent, high-quality neural cell cultures contain numerous complex components. Lot-to-lot variability in commercial neural culture supplements can result in experimental inconsistencies, driving some researchers to expend time and resources in screening lots before use.<sup>1</sup> STEMCELL Technologies rigorously pre-screens components of all media and supplements, and subjects finished products to extensive quality control testing.

STEMCELL's neural supplements can be used in conjunction with published protocols for a variety of neural cell culture applications, such as maintaining primary neurons, expanding neural stem cells, or differentiating pluripotent stem cells to neurons and glia.

## NeuroCult™ SM1 Neuronal Supplement

Primary neuronal culture is an important tool for many areas of neuroscience research; however, these cultures can be challenging. NeuroCult™ SM1 Neuronal Supplement (NeuroCult™ SM1, Catalog #05711) is based on Brewer's B27 supplement,<sup>2</sup> and optimized to more consistently support the culture of functional neurons. Neurons cultured using NeuroCult™ SM1 combined with BrainPhys™ Neuronal Medium (Catalog #05790) show increased cell survival when compared to the competitor formulation (Figure 1A). At 21 days *in vitro*, neurons cultured in NeuroCult™ SM1 are morphologically mature and show punctate expression of synaptic markers (Figure 1B).

Neural cultures derived from human pluripotent stem cells (hPSCs) require supplementation for long-term cellular health and survival. BrainPhys™ hPSC Neuron Kit (Catalog #05795) includes BrainPhys™ Neuronal Medium, N2 Supplement-A, NeuroCult™ SM1, and growth factors.

## Additional Cell Types and Protocols

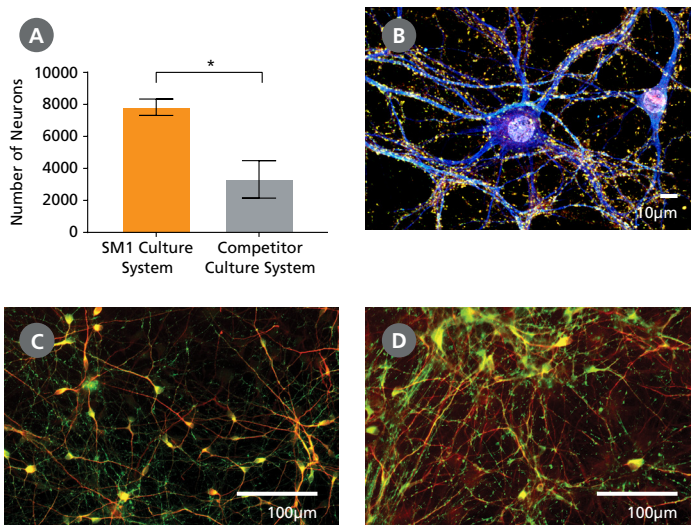
STEMCELL neural supplements were designed with the goal of long-term survival of neurons. For supplements used with additional cell types and stem cell differentiation applications, refer to page 2.

## Why Use STEMCELL Neural Supplements?

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

**STANDARDIZED.** Consistent culture conditions increase reproducibility among experiments.

**OPTIMIZED.** Formulations are optimized for culture, expansion, and differentiation of neural cells.



**Figure 1. Optimized Primary Neuronal Culture and hPSC-Derived Neural Progenitor Cell Differentiation Using the SM1 Culture System**

(A) Primary E18 rat cortical neurons were cultured in the SM1 culture system (BrainPhys™ Primary Neuron Kit, Catalog #05794) 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  $\pm$  95% CI;  $*p < 0.05$ ). (B) Primary E18 rat cortical neurons were cultured in the SM1 culture system (BrainPhys™ Primary Neuron Kit) by initially plating the neurons in NeuroCult™ Neuronal Plating Medium supplemented with NeuroCult™ SM1. On day 5, the cultures were transitioned to BrainPhys™ Neuronal Medium supplemented with NeuroCult™ SM1 by performing half-medium changes every 3 - 4 days. Neurons are phenotypically mature. Synapsin (green) co-localizes with PSD-95 (red) and is concentrated in discrete puncta distributed along the somata and dendritic processes, as defined by the dendritic marker MAP2 (blue). (C,D) Neural progenitor cells (NPCs) were generated from hES H9 cells using STEMdiff™ Neural Induction Medium (Catalog #05835) in an embryoid body-based protocol. NPCs were then cultured in BrainPhys™ Neuronal Medium with NeuroCult™ SM1 and N2 Supplement-A (Catalog #07152) for 14 (C) or 44 (D) days. Synapsin (green), MAP2 (red).

## NeuroCult™ SM1 Without Vitamin A

The presence of Vitamin A (or retinoic acid, retinyl acetate, etc.) in serum-replacement supplements can induce neural stem and progenitor cell differentiation, reducing progenitor culture purity and expansion efficiency. NeuroCult™ SM1 Without Vitamin A (Catalog #05731) can be used in conjunction with growth factors and your basal medium of choice for the expansion of neural stem and progenitor cells derived from the central nervous system (CNS) or from hPSCs.

## NeuroCult™ SM1 Without Antioxidants

Many cell types and especially neurons are selectively vulnerable to oxidative damage. NeuroCult™ SM1 Without Antioxidants (Catalog #05732) is our SM1 formulation with antioxidants removed (tocopherol, tocopherol acetate, superoxide dismutase, catalase, and glutathione). NeuroCult™ SM1 Without Antioxidants is suitable for studying the oxidative effects of chemicals or the beneficial effects of antioxidants (e.g. resveratrol, polyphenols, small molecules in general).

## NeuroCult™ SM1 Without Insulin

NeuroCult™ SM1 Without Insulin (Catalog #05733) can be used in protocols where the effects of insulin are being studied, or where insulin signaling may interfere with desired cellular function. NeuroCult™ SM1 Without Insulin can be used for cardiomyocyte differentiation protocols, as insulin has been shown to inhibit differentiation at early stages for these cell types.

## N2 Supplement-A

N2 Supplement-A (Catalog #07152) is based on Bottenstein's original N2 formulation<sup>3</sup> and can be used in neural culture protocols. These include protocols for i) neural induction of mouse or human embryonic stem (ES) cells or induced pluripotent stem (iPS) cells, ii) downstream differentiation of mouse or human ES or iPS-derived neural progenitor cells (NPCs) to specific neuronal and glial sub-types, and iii) differentiation of CNS-derived neural stem and progenitor cells.

## N2 Supplement-B

N2 Supplement-B (Catalog #07156) contains apo-transferrin in place of holo-transferrin, and is optimized for maintenance and expansion of neural progenitor cells in culture. Similar to N2 Supplement-A and NeuroCult™ SM1, N2 Supplement-B is subject to extensive raw material screening and quality testing to ensure reliability.

## Product Information

### Neural Supplements

PRODUCT	SIZE	CATALOG #
NeuroCult™ SM1 Neuronal Supplement	10 mL	05711
NeuroCult™ SM1 Without Vitamin A	10 mL	05731
NeuroCult™ SM1 Without Antioxidants	10 mL	05732
NeuroCult™ SM1 Without Insulin	10 mL	05733
N2 Supplement-A	5 mL	07152
N2 Supplement-B	5 mL	07156

### Related Products for Neural and Other Cell Culture Applications

PRODUCT	CATALOG #
BrainPhys™ Neuronal Medium	05790
BrainPhys™ Without Phenol Red	05791
BrainPhys™ Neuronal Medium and SM1 Kit	05792
BrainPhys™ Neuronal Medium N2-A & SM1 Kit	05793
BrainPhys™ Primary Neuron Kit	05794
BrainPhys™ hPSC Neuron Kit	05795
STEMdiff™ Cardiomyocyte Differentiation Kit	05010
STEMdiff™ Cardiomyocyte Maintenance Kit	05020
STEMdiff™ Cardiomyocyte Freezing Medium	05030

## References

1. Cressey D. (2009) Neuroscientists claim growing pains. *Nature* 459(7243):19.
2. 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.
3. Bottenstein JE. (1985) *Cell Culture in the Neurosciences.* (Bottenstein JE, Harvey A., eds.). Plenum Press: New York and London.

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