

# Neural Supplements

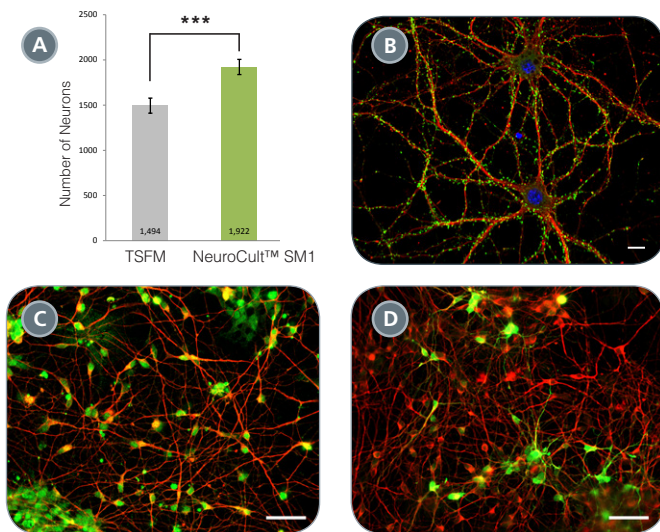
## For Consistent, High-Quality Neural Cell Cultures

### Advantages

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

**STANDARDIZED.** Standardized 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 NeuroCult™ SM1 and N2 Supplements**

(A-B) Primary E18 rat cortical neurons were cultured for 21 days in vitro (DIV) in NeuroCult™ SM1-supplemented NeuroCult™ Neuronal Basal Medium on poly-D-lysine-coated coverslips. (A) Significantly higher numbers of neurons were obtained when cultured in NeuroCult™ SM1, compared to a traditional serum-free formulation (TSMF; Neurobasal® Medium, 2% B-27® and 0.5 mM L-Glutamine;  $n = 25$ ; mean  $\pm$  95% CI; \*\*\* $p < 0.001$ ). (B) Neurons cultured in NeuroCult™ SM1 for 21 DIV are phenotypically mature. Synapsin (green) immunolabelling is concentrated in discrete puncta distributed along the somata and dendritic processes, as defined by MAP2 (red) staining. Nuclei were counterstained with DAPI (blue). Scale bar 10  $\mu$ m. (C-D) Neural induction of H9 cells was performed using STEMdiff™ Neural Induction Medium in an embryoid body-based protocol. Next, neural progenitor cells were differentiated in STEMdiff™ Neural Progenitor Basal Medium with 2% NeuroCult™ SM1 Supplement, 1% N2 Supplement-A and other growth factors. A mixed population of cortical neurons was generated (C: class III  $\beta$ -tubulin (red), FOXG1 (green); D: class III  $\beta$ -tubulin (red), gamma aminobutyric acid (GABA) (green)). Scale bar 50  $\mu$ m.

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 to screen lots before use.<sup>1</sup> STEMCELL Technologies rigorously pre-screens components of all media and supplements, and subjects our finished products to extensive quality control testing. In addition to our optimized, species-specific NeuroCult™ and STEMdiff™ kits, we offer a range of versatile neural supplements. These 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 to neurons and glia.

### Optimized Complete Kits

While our stand-alone neural supplements offer you versatility with minimal lot-to-lot variability, for optimal reproducibility, we recommend using our species- and application-specific NeuroCult™ and STEMdiff™ Kits.

### Supplements for Primary Neuronal Culture

Primary neuronal culture is an important tool for many areas of neuroscience research; however, these cultures can be challenging. NeuroCult™ SM Neuronal Supplements are based on Brewer's B27 supplement,<sup>2</sup> and optimized to more consistently support the culture of mature, functional neurons, while minimizing glial cell contamination (<1% GFAP). Neurons cultured using NeuroCult™ SM1 Neuronal Supplement show increased cell survival, neurite outgrowth and neurite branching, as compared to cultures using traditional serum-free medium (Figure 1A). At 21 days in vitro (DIV), neurons cultured in NeuroCult™ SM1 are morphologically mature and show punctate expression of synaptic markers (Figure 1B). NeuroCult™ SM2 Neuronal Supplement supports primary neurons in the absence of a culture substrate (e.g. poly-D-lysine or laminin), eliminating variability due to substrate quality, preparation or coating procedure.



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TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713 • INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM

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# Neural Supplements

For Consistent, High-Quality Neural Cell Cultures

## Supplements for Neural Stem Cell Expansion

The presence of Vitamin A (or retinoic acid, retinyl acetate, etc.) in serum-replacement supplements can induce neural stem and progenitor cell differentiation, reducing culture purity and expansion efficiency. NeuroCult™ SM1 Without Vitamin A 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 human pluripotent stem cells (hPSCs).

## Supplements for Neural Induction and Differentiation

Numerous published protocols for neural differentiation use Brewer's B27<sup>2</sup> supplement and/or Bottenstein's N2<sup>3</sup> supplement. 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. The minimal lot-to-lot variability of NeuroCult™ SM1 Neuronal Supplement, N2 Supplement-A (containing holo-transferrin) and N2 Supplement-B (containing apo-transferrin) can increase experimental reproducibility in many of these applications. For example, highly efficient and reproducible neuronal differentiation of hPSC-derived NPCs can be achieved using NeuroCult™ SM1 and N2 Supplement-A (Figure 1C-D). Alternatively, if you wish to control the levels of Vitamin A during differentiation, you may use NeuroCult™ SM1 Without Vitamin A and further supplement the medium accordingly.

## Complete Kits for Optimized Culture, Expansion and Differentiation

In addition to our versatile neural supplements, we provide an array of complete kits, designed to streamline your neural cell culture workflow. These kits are species-specific, optimized for each application, and are supplied with detailed technical protocols.

- NeuroCult™ SM1 and SM2 Neuronal Culture Kits for primary neuronal culture
- NeuroCult™ Proliferation Kits for CNS-derived neural stem and progenitor cell expansion
- NeuroCult™ Differentiation Kits for CNS-derived neural stem and progenitor cell differentiation
- STEMdiff™ Neural Induction Medium for generation of NPCs from human ES and iPS cells
- STEMdiff™ Neural Progenitor Medium for expansion of human ES- and iPS-cell derived NPCs

PRODUCT	SIZE	CATALOG #
NeuroCult™ SM1 Neuronal Supplement (50X)	10 mL	05711
NeuroCult™ SM1 Without Vitamin A (50X)	10 mL	05731
NeuroCult™ SM2 (Substrate-Independent) (50X)	2 mL	05721
N2 Supplement-A (100X)	5 mL	07152
N2 Supplement-B (100X)	5 mL	07156

**Table 1.** Neuronal Supplements for Culture, Expansion and Differentiation

PRODUCT	SIZE	CATALOG #
NeuroCult™ SM1 Neuronal Culture Kit	1 kit	05712
NeuroCult™ SM2 Substrate-Independent Neuronal Culture Kit	1 kit	05722
NeuroCult™ NS-A Proliferation Kit (Human)	1 kit	05751
NeuroCult™ Proliferation Kit (Mouse)	1 kit	05702
NeuroCult™ NS-A Proliferation Kit (Rat)	1 kit	05771
NeuroCult™ NS-A Differentiation Kit (Human)	1 kit	05752
NeuroCult™ Differentiation Kit (Mouse)	1 kit	05704
NeuroCult™ NS-A Differentiation Kit (Rat)	1 kit	05772
STEMdiff™ Neural Induction Medium	250 mL	05835
STEMdiff™ Neural Progenitor Medium	1 kit	05833

**Table 2.** Complete Kits for Optimized Culture, Expansion and Differentiation of Neural Cells

## References

1. Cressey D. (2009) Neuroscientists claim growing pains. *Nature* 459(7243):19.
2. Brewer GJ, Torricelli JR, Evege EK, Price PJ. (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.