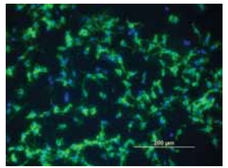
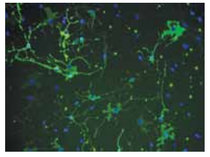


Figure 8. Retention of undifferentiated state. Rat GPCs cultured in GIBCO® basal medium supplemented with StemPro® Neural Supplement were stained by indirect immunofluorescence for the cell-surface marker A2B5 (green). Nuclei were stained with DAPI (blue). Cells were maintained in the undifferentiated state in medium supplemented with StemPro® Neural Supplement.

A



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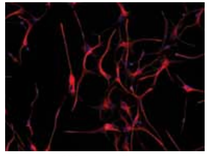

Figure 9. Human GPCs retain differentiation potential. Human GPCs were cultured in KnockOut™ DMEM/F-12 basal medium supplemented with StemPro® Neural Supplement and were differentiated into **(A)** oligodendrocytes and **(B)** astrocytes. Oligodendrocytes were stained with biomarker of GalC (green), and astrocytes were stained with biomarker of GFAP (Red). Nuclei were labeled with DAPI.

Retains multipotent differentiation potential of GPCs

Human GPCs grown in StemPro® Neural Supplement retain their differentiation potential into oligodendrocytes and astrocytes (Figure 9). Figure 10 shows the rat GPCs after proliferation differentiated into oligodendrocytes. Components required for complete media for GPCs are summarized in Table 2.

Supports survival of differentiated neurons and enables serum-free differentiation of PCs into astrocytes

The application of StemPro® Neural Supplement can be extended to support survival of differentiated neurons. StemPro® Neural Supplement can support differentiated neurons when used with Neurobasal® Medium or other basal formulations. The performance of StemPro® Neural Supplement was tested side-by-side with standard B-27® supplement and NS21. The results showed that the performance of StemPro® Neural Supplement at day 4 and day 8 was comparable with that of the standard B-27® supplement and superior to NS21 [3]. Hence, its use can be expanded to the culture system where serum-free supplements such as B-27® and NS21 are desired.

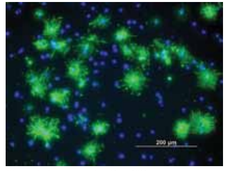

Figure 10. Rat GPCs retain differentiation potential. Rat GPCs were cultured in GIBCO® basal medium supplemented with StemPro® Neural Supplement and differentiated further into oligodendrocytes. Oligodendrocytes were stained with the biomarker GalC (green), and nuclei were labeled with DAPI.

Table 2. Components required to culture GPCs.

Product	Stock concentration	Final concentration	For 100 mL	For 500 mL
StemPro® Neural Supplement	50X	1X	2 mL	10 mL
KnockOut™ DMEM/F-12	1X	1X	97 mL	485 mL
GlutaMAX™-I Supplement	100X	1X	1 mL	5 mL
bFGF	20 µg/mL	20 ng/mL	100 µL	500 µL
PDGF-AA	10 µg/mL	10 ng/mL	100 mL	500 µL

StemPro® Neural Supplement enables serum-free differentiation of neural progenitor cells into astrocytes. When used with DMEM medium with cytokines, serum-free differentiation into astrocytes can be obtained without the undefined factors found in other serum-containing medium.

References

1. Rao MS (2005) *Neural Development and Stem Cells, Second Edition*. Totawa (NJ): Humana Press. pp 1–454.
2. Doering LC (2009) *Protocols for Neural Cell Culture, Fourth Edition*. Totawa (NJ): Humana Press.
3. Brewer GJ, Torricelli JR, Evege EK et al. (1993) *J Neurosci Res* 35:567.

Ordering information

Product	Quantity	Cat. No.
StemPro® Neural Supplement	10 mL	A1050801
KnockOut™ DMEM/F-12	500 mL	12660012
FGF-Basic Recombinant Human	10 µg	PHG0024
EGF Recombinant Human	10 µg	PHG0314
PDGF-AA Recombinant Human	10 µg	PHG0035
GlutaMAX™-I Supplement	100 mL	35050-061
CELLstart™	2 mL	A1014201

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Serum-free supplement for neural stem and progenitor cells

StemPro® Neural Supplement

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Serum-free growth and expansion of neural stem cells (NSCs) and neural progenitor cells (PCs)

StemPro® Neural Supplement

- → Supports superior expansion and retains multipotent differentiation potential of NSCs
- → Provides superior proliferation of neural PCs and retains their undifferentiated marker expression
- → Application can be extended to support differentiated neurons and serum-free differentiation of neural PCs towards astrocytes

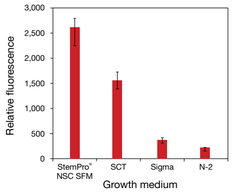

Figure 1. Superior human NSC expansion is achieved in basal medium supplemented with StemPro® Neural Supplement over competitors' serum-free NSC media. KnockOut™ DMEM/F-12 supplemented with StemPro® Neural Supplement demonstrates superior cell expansion capacity compared with standard N-2–supplemented and competitor neural stem cell media formulations. Proliferation of hNSCs cultured in StemPro® Neural Supplement, competitor SCT medium, Sigma medium, and N-2–supplemented medium was measured. ESC-derived hNSCs were seeded at 1 x 10^4 cells per well in CELLstart™ Substrate–coated 96-well plates for 3 days in respective media. Indirect cell count was obtained with the CyQUANT® Proliferation Assay Kit (Cat. No. C35006), and data show mean relative fluorescence units of stained cells (n = 6).

In recent years, there has been an increasing scientific interest in the study of neural stem and progenitor cells due to their important roles in neurogenesis. Recent advances point to the potential of NSCs and neural PCs as effective therapies for neurodegenerative diseases, including dopaminergic neural progenitor cells for Parkinson's disease and oligoprogenitor cells for amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) [1].

Since the first isolation of NSCs, scientists initially relied on serum-containing media for NSC culture and expansion. NSCs as well as neural PCs differentiate into neurons and glial cells in serum-containing media. While serum-free alternatives such as the GIBCO® Neurobasal® Medium/N-2 combination and serum-free media from other companies alleviate problems associated with serum use [2], these systems often resulted in suboptimal cell expansion. To address the need for an optimized serum-free solution, we developed StemPro® Neural Supplement for superior proliferation of mammalian neural stem and progenitor cells.

Supports superior proliferation and retains multipotent differentiation potential of NSCs

Neural stem cells are self-renewing, multipotent stem cells that can differentiate into neurons, oligodendrocytes, and astrocytes. As NSCs are a very small fraction of the total CNS cell population, expansion is critical to generate sufficient cells to study differentiation pathways and explore downstream clinical applications. NSCs can be passaged only a limited number of times before exhibiting reduced proliferation and differentiation potential. Currently, NSCs are cultured in serum-containing media or conventional media supplemented with N-2 neural growth factor, which contains undefined components and can result in lot-to-lot variability. StemPro® Neural Supplement and KnockOut™ DMEM/F-12 maximizes the total hNSC yield per passage (Figure 1) compared to other competitor media. Moreover, StemPro® Neural Supplement supports growth and expansion of NSCs grown in both adherent and neurosphere suspension cultures (Figure 2).

Human NSCs grown with StemPro® Neural Supplement retain their phenotypic marker expression. Figure 3 shows that hNSCs retain the expression of neural stem cell markers Nestin and Sox2 and proliferation marker Ki67.

hNSCs are defined by the ability to differentiate into three distinct lineages—neurons, oligodendrocytes, and astrocytes. StemPro® Neural Supplement maintains the multipotent differentiation capabilities of stem cells and the ability to drive hNSCs down the desired lineage to meet specific experimental requirements (Figure 4). For NSC culture, StemPro® Neural Supplement is available as a complete media called StemPro® NSC SFM (for components, see Table 1).

Supports superior proliferation of neural progenitor cells and retains their multipotent differentiation potential

Due to the scarce availability of glial precursor and neuronal progenitor cells, *in vitro* expansion of PCs has been a challenge. For example, glial precursor cells (GPCs) are restricted progenitors; the majority of their downstream progeny are oligodendrocytes and astrocytes. Because of their capacity to generate oligodendrocytes and astrocytes, GPCs can be used for neuroscience studies as well as stem cell differentiation, tissue engineering, cell and genetic therapy, and transplantation experiments. StemPro® Neural Supplement enables superior expansion of GPCs resulting in more than 50-fold increases in cell number (Figure 5). GPCs grown in StemPro® Neural Supplement retain their progenitor status after P3 (passage 3), compared to GPCs grown in ScienCell™ OPC medium (competitor medium), where no proliferation was apparent but differentiated cells such as astrocytes appeared (Figure 6).

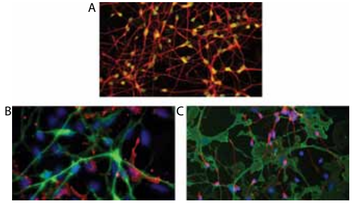

Figure 4. Differentiation potential of hNSCs cultured in KnockOut™ DMEM/F-12 basal medium supplemented with StemPro® Neural Supplement. hNSCs were cultured in media supplemented with StemPro® Neural Supplement and were differentiated into neurons and glial cells. **(A)** Neurons were labeled with an anti-HuC/D antibody (green) and an anti-Dcx antibody (red). **(B)** Cells with an oligodendrocyte lineage were labeled with an anti-GalC antibody (red). Cell nuclei were labeled with DAPI (blue), and neurons were labeled with an anti-Dcx antibody (green). **(C)** Cells with an astrocyte lineage were labeled using an anti-CD44 antibody (green). Cell nuclei were labeled with DAPI (blue), and neurons were labeled with an anti-Dcx antibody (green).

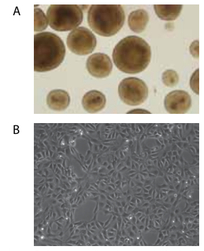

Figure 2. Stable proliferation of hNSCs in StemPro® Neural Supplement enables culturing of both adherent and suspension culture systems. StemPro® Neural Supplement provides the flexibility to culture hNSCs for several passages, maintaining multipotent characteristics as either neurosphere **(A)** or adherent culture **(B)**. hNSCs were derived from hESCs cultured in KnockOut™ DMEM/F-12 supplemented with StemPro® Neural Supplement for 7 passages on CELLstart™ Substrate. Tertiary neurospheres were isolated from fetal tissue cultured in NSC SFM.

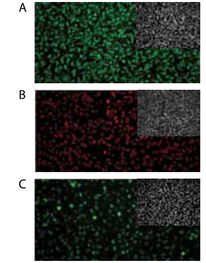

Figure 3. Phenotype marker expression of hNSCs cultured in StemPro® Neural Supplement. hNSCs express normal phenotypic markers **(A)** Nestin, **(B)** Sox2, and proliferation marker **(C)** Ki67. There was no sign of remnant hESC (Oct4). The inset image in each panel shows the staining pattern given by the nuclear stain DAPI.

Retains multipotent phenotype of human and rat GPCs

The undifferentiated state of GPCs cultured in StemPro® Neural Supplement was determined based on the phenotypic marker expression of PDGFRa, Olig2, and NG2 markers. Figure 7 shows human GPCs cultured in StemPro® Neural Supplement retaining their undifferentiated state.

Upon expansion in media supplemented with StemPro® Neural Supplement, more than 80% of rat GPCs retain expression of undifferentiated phenotypic marker A2B5. Figure 8 shows rat GPCs cultured in StemPro® Neural Supplement retaining their undifferentiated state.

Table 1. Components required for NSC culture.

Product	Stock concentration	Final concentration	For 100 mL	For 500 mL
StemPro® Neural Supplement	50X	1X	2 mL	10 mL
KnockOut™ DMEM/F-12	1X	1X	97 mL	485 mL
GlutaMAX™-I Supplement	100X	1X	1 mL	5 mL
bFGF	20 µg/mL	20 ng/mL	100 µL	500 µL
EGF	20 µg/mL	20 ng/mL	100 µL	500 µL

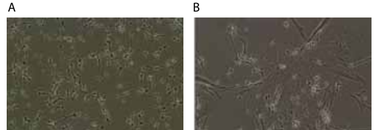

Figure 6. Superior proliferation of GPCs is achieved using StemPro® Neural Supplement compared with ScienCell™ OPC SFM. GPCs were cultured either in GIBCO® basal medium (with StemPro® Neural Supplement) **(A)** or in ScienCell™ OPC medium (competitor medium) **(B)**. During the 3-week culture period, cells cultured in GIBCO® medium **(A)** could be passaged for 3 times, resulting in 50-fold more cells while retaining their phenotype. However, cells cultured in ScienCell™ OPC medium **(B)** did not result in an increase in cell number but resulted in a loss of normal phenotype and differentiation to downstream lineages such as astrocytes (large cell bodies).

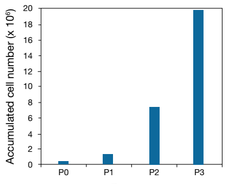

Figure 5. Superior proliferation of GPCs. GPCs were cultured in GIBCO® basal medium supplemented with StemPro® Neural Supplement. Every 7 days, cells were harvested and re-plated for further proliferation, and the cell number was documented. After passage 3, accumulated cell number was more than 50-fold higher than starting cell numbers.

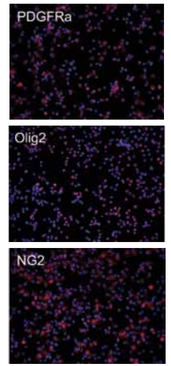

Figure 7. Phenotype marker expression of human GPCs cultured in GIBCO® basal medium supplemented with StemPro® Neural supplement. GPCs were labeled with GPC biomarkers PDGFRa, Olig2, and NG2. The above data reveal the ability of StemPro® Neural Supplement to support GPCs to retain their undifferentiated status.