StemPro® NSC SFM maintains the multipotent differentiation capabilities of hNSCs

hNSCs are defined by the ability to differentiate into three distinct lineages—neurons, oligodendrocytes, and astrocytes. StemPro® NSC SFM is a robust serum-free neural stem cell medium that maintains the multipotent differentiation capabilities of stem cells and the ability to drive hNSCs down the desired lineage to meet specific experimental requirements (Figure 5).

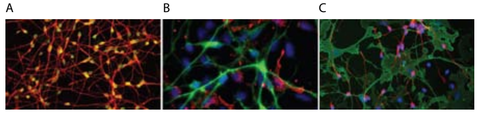

Figure 5—Differentiation potential of hNSCs cultured in StemPro® NSC SFM. hNSCs were cultured in StemPro® NSC SFM and were differentiated into neurons and glial cells. **(A)** Neurons were labeled with an anti-HuC/D antibody (green) and an anti-Dcx antibody (red). **(B)** Cells with an oligodendrocyte 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).

hNSCs grown in StemPro® NSC SFM exhibit normal neural cell functionality

Following differentiation of hNSCs into neurons using StemPro® NSC SFM, the cells exhibited normal electrophysiological behavior in response to a panel of neurotransmitters (acetylcholine, glutamate, norepinephrine, and ATP), as assessed by indirect measurement of calcium concentration (Figure 6).

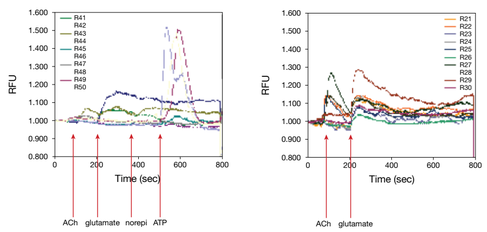

Figure 6—hNSCs differentiated to neurons in StemPro® NSC SFN maintain expected physiological properties. Cells were exposed to various neurotransmitters, and the responses were evaluated by indirect measurement of calcium concentration (fluo-4 emission intensity). Differentiated neurons cultured in StemPro® NSC SFM retain physiologically reactive responses to the neurotransmitters acetylcholine, glutamate, norepinephrine, and ATP. Each R number with accompanying colored line represents an individual cell body being evaluated in this experiment. The arrows indicate points in the experiment when a neurotransmitter was added and a reading was taken.

Ordering information

Product	Quantity	Cat. no.
StemPro® NSC SFM*	1 kit	A10509-01
CELLstart™ Humanized Substrate for Cell Culture	2 ml	A10142-01

* StemPro® NSC SFM is shipped as separate components which have separate storage requirements. The StemPro® NSC SFM Supplement is not sold individually. KnockOut™ DMEM F-12 Basal Medium (500 ml; store in the dark at 2 to 8°C); FGF Basic Recombinant Human and EGF Recombinant Human Growth Factors (10 µg; store at 2 to 8°C, desiccated); StemPro® NSC SFM Supplement (10 ml; store frozen at –5 to –20°C).

CELLstart™ defined, xeno-free cell culture substrate for stem cells
To learn more, visit www.invitrogen.com/stemcell/cellstart.

Advance your NSC research with the first serum-free medium for hNSCs.
To learn more, ask your Invitrogen Account Manager or visit www.invitrogen.com/stempro/nsc for product information and protocols.

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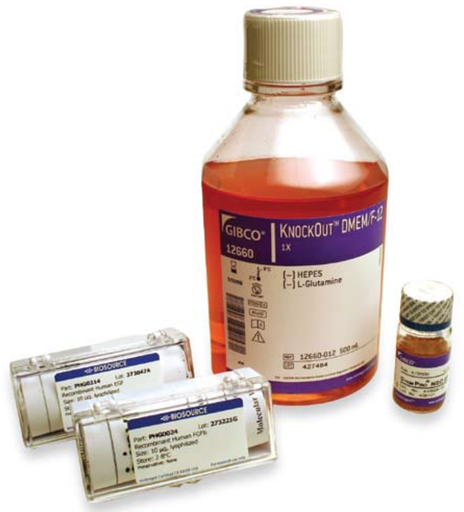
Superior NSC expansion with serum-free medium

StemPro® NSC SFM:
Serum-Free Human Neural Stem Cell Culture Medium



Specially formulated for serum-free growth and expansion of human neural stem cells

StemPro® NSC SFM

- → Superior expansion of human neural stem cells (hNSCs) derived from either embryonic stem cells or fetal tissue
- → Versatility to support long-term growth and expansion of both adherent and neurosphere suspension cultures
- → Maintain normal hNSC multipotency and phenotype/karyotype
- → hNSCs grown in StemPro® NSC SFM maintain the potential to differentiate into physiologically active neurons and glial cells
- → Better batch-to-batch consistency, with each lot produced under cGMP and qualified using an hNSC performance assay
- → Little or no adaptation required from serum-supplemented medium

Serum-free medium for hNSC culture

Human neural stem cells (hNSCs) are self-renewing, multipotent stem cells of the nervous system that can differentiate into neurons, oligodendrocytes, and astrocytes. Multipotent neural stem cells reside in the subventricular zone (SVZ) and the hippocampus in the fetal and adult brain. hNSCs can be isolated from these tissues,[1-3] but often there is limitation in their differentiation potential, with cells tending to exhibit characteristics similar to the tissue from which they were derived.[4-6] Alternatively, hNSCs can be derived from embryonic stem cells,[7,8] which possess greater flexibility in which lineage the cells can be driven toward.[9,10] Because of their ability to generate neurons and glial cells, hNSCs are a valuable source not only for basic neuroscience but also for downstream clinical applications aimed at treating neurodegenerative disease or neurological disorders.

Found in specific regions of the CNS, hNSCs have only recently been identified. As a result, technical limitations still hamper the manipulation of hNSCs *in vivo*, and a stable *in vitro* culture system is needed to provide adequate numbers of stem cells for use in research and potential clinical applications. Currently hNSCs are cultured in serum-supplemented media or in conventional media supplemented with N2 neural growth factor. However, serum contains undefined components that can contribute to lot-to-lot variability and can compromise studies involving pathway and mechanism analysis. Defined N2 medium suffers from the limitation that it does not allow stable proliferation of hNSCs. Therefore, to advance research in this area, a reliable serum-free medium designed for optimal growth of hNSCs is required. StemPro® NSC SFM supports hNSC maintenance and efficient differentiation into the three neural lineages for further characterization studies.

StemPro® NSC SFM was developed to enable serum-free growth and expansion of hNSCs in either adherent or neurosphere suspension cultures. Using StemPro® NSC SFM, hNSCs can be expanded for multiple passages while maintaining their multipotent phenotype (i.e., ability to differentiate into neuronal, oligodendrocyte, and astrocyte lineages). StemPro® NSC SFM is a cGMP-manufactured medium that delivers the quality and consistency needed to optimize your hNSC culture.

Superior hNSC expansion and versatility to grow both adherent and neurosphere suspension cultures

As hNSCs comprise a very small fraction of the total CNS cell population, expansion is critical to generate sufficient cells to study differentiation pathways and explore the downstream clinical applications of hNSCs. hNSCs can be passaged only a limited number of times before exhibiting reduced proliferation and differentiation potential. Maximizing the total hNSC yield per passage is therefore essential (Figure 1). In addition, StemPro® NSC SFM offers the flexibility to grow both adherent and neurosphere suspension cultures (Figure 2).

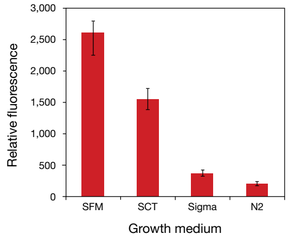


Figure 1—Superior hNSC expansion is achieved using StemPro® NSC SFM compared with competitors' serum-free NSC media. StemPro® NSC SFM demonstrates superior cell expansion capacity compared with standard N2-supplemented and competitors' neural stem cell media. Proliferation of hNSCs cultured in Invitrogen StemPro® NSC SFM (SFM), competitor SCT medium (SCT), Sigma medium (Sigma), and N2-supplemented medium (N2) was measured. ESC-derived hNSCs were seeded at 1 x 10^4 cells per well in CELLstart™ substrate–coated 96-well plates and grown for 3 days in respective media. Fluorescence data were obtained from stained cells (n = 6) using the CyQUANT® NF Cell Proliferation Assay Kit (Cat. no. C35006); cell counts were extrapolated from the fluorescence measurements.

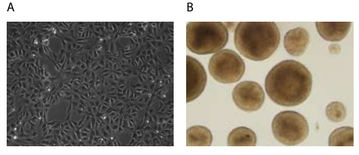


Figure 2—StemPro® NSC SFM promotes stable proliferation of hNSCs in both adherent and suspension culture systems. StemPro® NSC SFM provides the flexibility to culture hNSCs for several passages, maintaining multipotent characteristics of either **(A)** adherent or **(B)** neurosphere cultures. hNSCs were derived from hESCs cultured in NSC SFM for 7 passages on CELLstart™ substrate. Tertiary neurospheres were isolated from fetal tissue cultured in NSC SFM.

StemPro® NSC SFM maintains multipotency and normal phenotype/karyotype

Markers for the accurate identification of neural stem cells continue to be defined and expanded. At present, identifying neural stem cells relies on testing for a combination of positive and negative phenotypic expression markers.[11,12] As a self-renewing population, hNSCs express the proliferation marker Ki67, and the CD133[13] cell-surface protein can also be used for cell sorting. Nestin is a class VI intermediate filament protein expressed predominantly in neural stem cells. Sox1 and Sox2 are HMG box transcription factors reportedly expressed in neural stem cells as well. Neurofilament, NCAM, MAP2, β III tubulin, NeuN, HuC/D, and Dcx are expressed in neuronal progenitors or fully differentiated neurons.[14,15] GalC, NG2, O4, myelin basic protein, CNPase, and RIP are expressed in oligodendrocyte progenitors or oligodendrocytes.[16] CD44, GFAP, and S-100 protein are expressed in astrocyte progenitors or fully differentiated astrocytes.[17]

Human neural stem cells grown in StemPro® NSC SFM retain normal expression of phenotypic markers, including nestin and Sox2 as well as the proliferation marker Ki67, through several passages (Figure 3). StemPro® NSC SFM also maintains normal hNSC karyotype in culture (Figure 4).

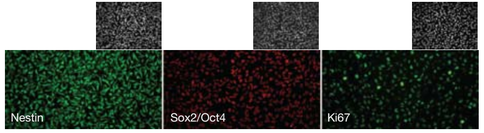


Figure 3—Phenotypic marker expression of hNSCs cultured in StemPro® NSC SFM. hNSCs expressed normal phenotypic markers (nestin, Sox2) and a proliferation marker (Ki67) through passage 17. There was no sign of remnant hESC (Oct4). The smaller images show nuclear DAPI staining.

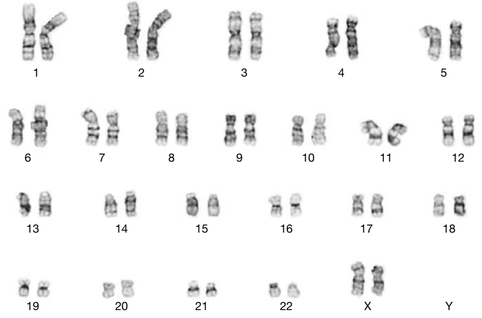


Figure 4—hNSCs grown in StemPro® NSC SFM retain a normal karyotype through passage 5.