

Enhanced expansion of neural stem cells using a heat stable bFGF

Introduction

Neural stem cells (NSCs) are widely used for the study of neural development and, upon differentiation into neural cell types, are also used to study neurodegenerative diseases and their potential treatments. In order to maintain multipotency and proliferation of NSCs, it is often necessary to supplement growth media with basic fibroblast growth factor (bFGF). However, native bFGF is not stable at standard culture conditions (37°C, 5% CO₂) and loses activity quickly (Figure 1A). It is common to compensate for this loss of activity by changing the medium every 1–3 days. Even with this adjustment, native bFGF starts degrading shortly after each media change. This introduces variability to the system and is nonoptimal for the expansion and maintenance of multipotent of NSCs.

Gibco™ Heat Stable Recombinant Human bFGF Protein (HS bFGF, Cat. No. PHG0360) offers a solution to this problem. Cells respond to Gibco HS bFGF just as they would to the native protein. Whereas native bFGF degrades over the course of an experiment, HS bFGF is engineered to maintain bioactivity at 37°C (Figure 1B). In this application note, we demonstrate the ability of HS bFGF to deliver enhanced expansion of multipotent NSCs as compared to the native protein, without the need for media changes between passages.

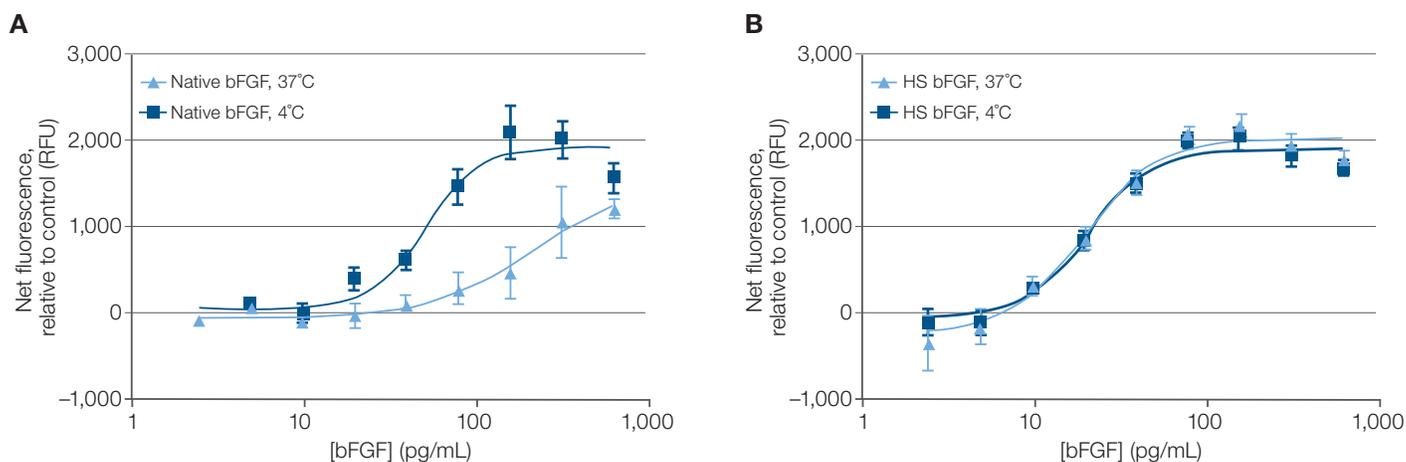


Figure 1. HS bFGF is engineered to sustain bioactivity at standard culture conditions. Activity of native bFGF (A) and HS bFGF (B) after 72 hours at 37°C (light blue), as compared against the control (dark blue) at 4°C. Activity measured by mouse BALB/3T3 proliferation using Invitrogen™ PrestoBlue™ Cell Viability Reagent, reported as the mean ± standard error of the mean (SEM).

Experimental workflow

In this study, we compared human embryonic stem cell-derived NSC proliferation (i.e., cell count at passage) using HS bFGF and native bFGF. A concentration of 20 ng/mL bFGF was used in NSC growth medium with medium change carried out only upon passage (every 3–4 days) (Figure 2). HS bFGF and the native human bFGF control were added into Gibco™ KnockOut™ DMEM/F-12 medium supplemented with Gibco™ StemPro™ Neural Supplement

and GlutaMAX™ Supplement. The NSCs were seeded at approximately 4×10^4 cells/cm² for a three-day split and 2×10^4 cells/cm² for a four-day split. Viability measurements and cell counts were collected at each passage, for six passages. From these data, the number of population doublings at each passage was calculated. At the fifth passage, NSCs were plated and fixed for immunostaining. Multipotency was verified by SOX1 expression.

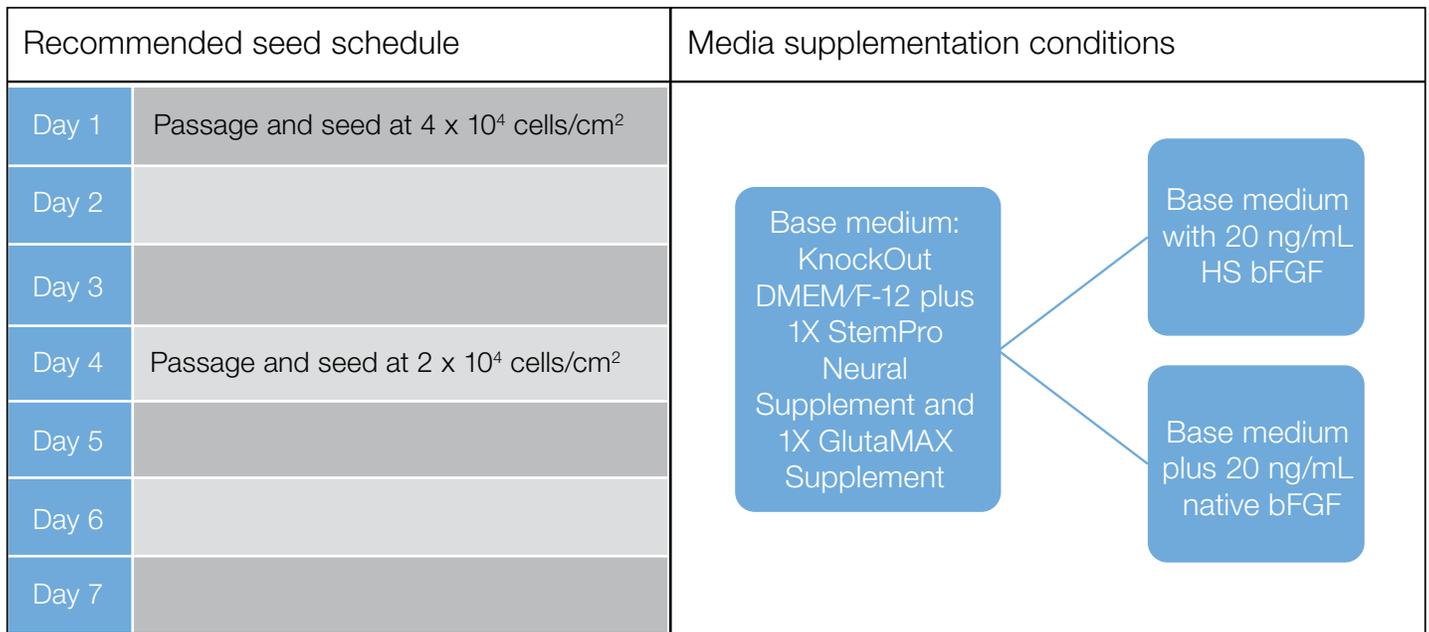


Figure 2. Workflow and experimental media supplementation for NSC expansion.

Results

NSC cultures grown in the presence of HS bFGF demonstrated improved expansion relative to those grown in native bFGF (Figure 3). This may be attributed to the stability of HS bFGF, as opposed to the varying bioactivity levels of native bFGF that is caused by heat-induced degradation of the protein. As with native bFGF, HS bFGF maintained the expected NSC morphology as well as expression of the multipotency marker SOX1 after five passages (Figure 4). Taken together, these data suggest that HS bFGF enhances the rate of expansion of NSCs while maintaining multipotency, even under limited feeding conditions.

Conclusions

Heat Stable Recombinant Human bFGF Protein supports enhanced flexibility and superior expansion in the culture of NSCs. Replacement of native protein with HS bFGF not only allows for reduced culture feeds but also results in a significant increase in NSC growth, yielding 40% more multipotent NSCs after 21 days in culture.

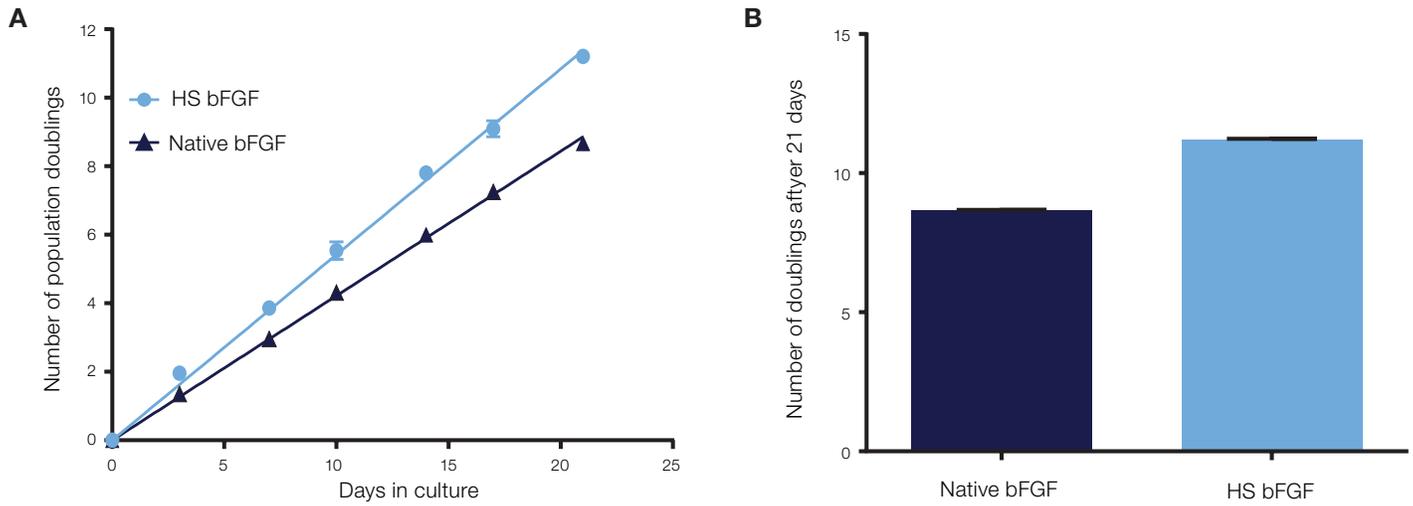


Figure 3. HS bFGF enhances expansion of NSCs, relative to native bFGF. Number of population doublings at each passage (**A**) and the resulting number of population doublings at the last passage (**B**), reported as the mean \pm standard deviation (SD).

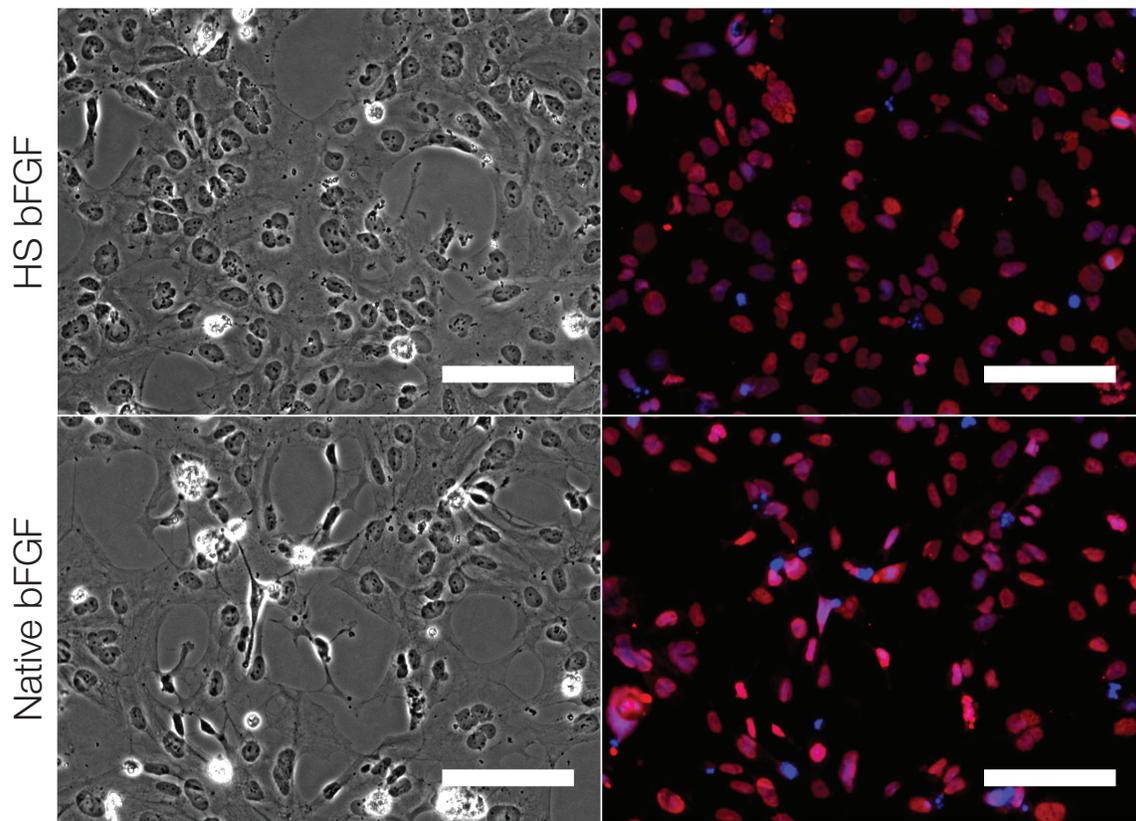


Figure 4. HS bFGF protein maintains the morphology and SOX1 expression of multipotent NSCs. Phase-contrast (left) and immunostained (right) photomicrographs of NSCs. Immunostaining for NSC multipotency marker SOX1 (red) with nuclear (DAPI) counterstain (blue). Scale bars = 100 μ m.

Ordering information

Product	Cat. No.
Heat Stable Recombinant Human bFGF Protein, 5 µg	PHG0367
Heat Stable Recombinant Human bFGF Protein, 50 µg	PHG0368
Heat Stable Recombinant Human bFGF Protein, 100 µg	PHG0369
Heat Stable Recombinant Human bFGF Protein, 500 µg	PHG0360
KnockOut DMEM/F-12	12660012
StemPro Neural Supplement	A1050801
GlutaMAX Supplement	35050061
DPBS, no calcium, no magnesium	14190
StemPro Accutase Cell Dissociation Reagent	A1110501
Nunc EasYFlask Cell Culture Flasks	156340
Nunc Cell Culture–Treated Multidishes	142485
Human Neural Stem Cell Immunocytochemistry Kit	A24354
EVOS FL Auto 2 Imaging System	AMAFD2000

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