proteintech®
Antibodies | ELISA kits | Proteins

Humankine® product line
HUMANZYME
Now part of Proteintech Group®

White Paper

Thermostable FGF Basic Supports a 2-- Day Feeding Schedule As Shown by Biochemical and Cell Culture Analysis

Mark Azam, Ph.D.
VP -- R&D and
Manufacturing

Patricia Ahrweiler, Ph.D.
Sr. Marketing Consultant

Summary

FGF basic is a required component of stem cell culture media for maintaining cells in an undifferentiated state. Because FGF basic is unstable, daily media changes are needed. HumanZyme has developed a thermostable FGF basic (FGFbasic-- TS) that supports a 2-- day media change schedule, so no media changes are required over a weekend. This thermostable FGF basic was more stable than FGF basic in biochemical studies, and maintained cell growth, pluripotency and differentiation potential with a 2-- day feeding schedule, as evaluated with pluripotency markers, karyotyping and a reporter gene assay.

Introduction

Fibroblast growth factors (FGF) are a family of heparin-- binding secreted proteins that stimulate cell proliferation and differentiation in a wide variety of tissues. FGFs play important roles in diverse biological functions both *in vivo* and *in vitro*, including mitogenesis, cellular migration, differentiation, angiogenesis, and wound healing.

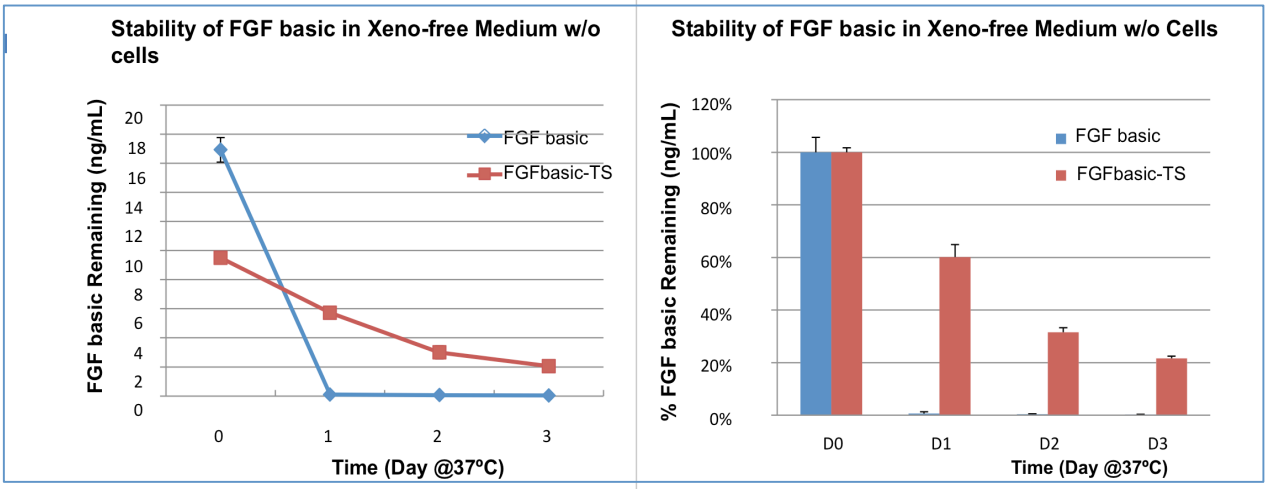
Human embryonic stem cell (hESC) cultures require FGF basic (also known as FGF-- 2 or bFGF) in cell culture media to remain in an undifferentiated and pluripotent state, although the specific mechanism for this is unknown. Since FGF basic is unstable, daily media changes with fresh addition of FGF basic have been required, which can be costly, tedious and time consuming. HumanZyme has developed a thermal stable FGF basic (FGFbasic-- TS) that supports a 2-- day instead of daily media change protocol. A 2-- day protocol eliminates the need for media changes over the weekend, a welcome improvement in current cell culture protocols for stem cell researchers.

Thermostable FGF basic (FGFbasic-- TS) was engineered for enhanced stability in culture media, without modification of its biological function. The 154 amino acid, 17 kDa, non-- glycosylated monomer cytokine is expressed in HumanZyme's proprietary human (HEK293) cell line developed for production of authentic human recombinant proteins. In this White Paper, biochemical and cell culture growth and expansion studies were performed to show this thermostable FGF basic can be used in a defined, xeno-- free, cGMP-- compatible cell culture system with a 2-- day feeding schedule, and can maintain good cell growth activity, pluripotency and differentiation potential.

Biochemical Characterization

Stability in Cell Culture Media Without Cells

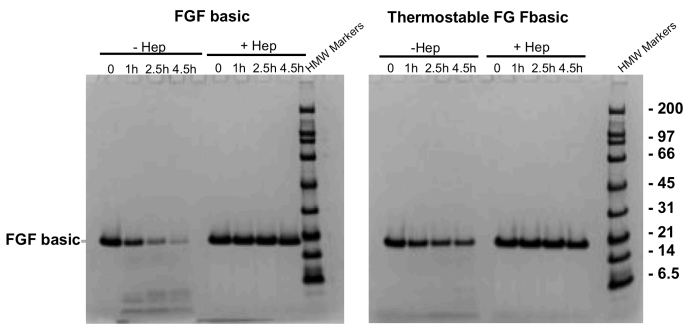
The stability of thermostable FGF basic (HumanZyme) and FGF basic (*E. coli* produced) in cell culture media without cells was evaluated by incubating both proteins at 9 ng/mL (FGFbasic-- TS) and 17 ng/mL (FGF basic) in xeno-- free, chemically defined cell culture media at 37˚C without cells. The protein concentration was determined by ELISA each day for 3 days.



Results are shown in Figure 1. After one day of incubation at 37 ˚C, FGF basic was undetectable, while FGF basic-- TS was present at levels 60%, 35%, and 20% of its starting concentration at days 1, 2, and 3 respectively. These results suggest thermostable FGF basic degrades roughly 3x more slowly than FGF basic.

Resistance to Enzymatic Digest

Figure 2. Trypsin digestion of FGF basic and thermostable FGF basic



HMW BioRad protein standard (1uL) and FGF basic or thermostable FGF basic (5ug) were loaded onto a 4-- 12% Nu-- PAGE Bis-- Tris gel. The gel was electrophoresed under non-- reducing conditions with MOPS running buffer and stained with SafeStain.

Next, resistance of thermostable FGF basic and FGF basic towards trypsin enzymatic proteolysis was determined. Both proteins were subjected to a tryptic digest then analyzed on a SDS-- PAGE gel, without and with Heparin to stabilize against degradation by trypsin.

As shown in Figure 2, FGF basic was significantly degraded after 4.5 hours, while thermostable FGF basic protein levels were only slightly reduced.

Thermostable FGF basic appears to have enhanced biochemical stability compared to FGF basic. Thermostable FGF basic was more stable in cell culture media, and more resistant towards proteolytic degradation compared to FGF basic.

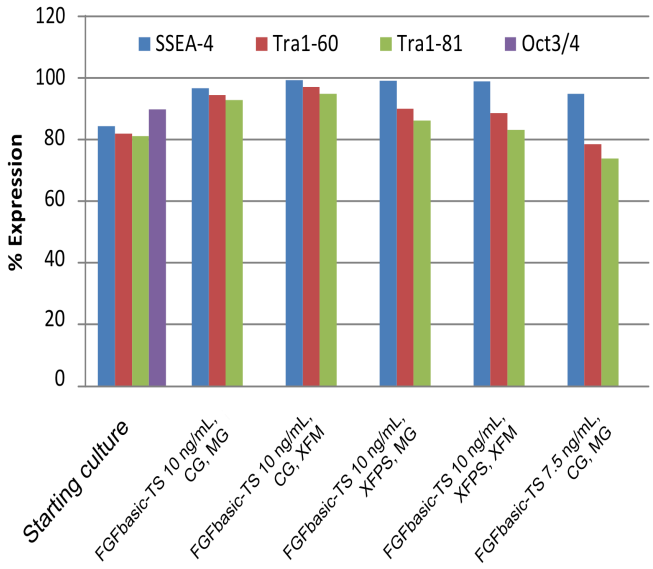
Cell Culture Analysis of Thermostable FGF Basic

Analysis of Pluripotency Markers

The hallmark of stem cells is their pluripotency, or the ability to give rise to all cell types. Molecular markers are one method to characterize the status of a pluripotent stem cell by the expression of these markers over passaging. Thermostable FGF basic was evaluated for effective maintenance of pluripotency markers using a 2-- day feeding schedule in human embryonic stem cells (hESC) and human induced pluripotent stem cells (iPSC). Thermostable FGF basic was added at 7.5 or 10 ng/mL.

Human cell lines were cultured in xeno-- free, chemically defined media containing either a xeno-- free chemically defined matrix (XFM) or Matrigel
(MG). Cells were either passaged using xeno-- free, non-- enzymatic passaging solution (XFPS) or a collagen-- based reagent (CG).

Figure 3a. Pluripotency markers in hESC cultures maintained with thermostable FGF basic, in the starter culture and after 10 passages



Pluripotency cell surface markers SSEA 4, Tra1-- 60, and Tra1-- 81 were analyzed by flow cytometry for hESC and iPSC cultures grown in media containing 7.5 or 10 ng/mL thermostable FGF basic (FGFbasic-- TS) on the starting culture and after the 10th split, for all culture conditions. Transcription factor Oct 3/4 was only analyzed on the starter culture.

As shown in Figure 3a and 3b, overall, pluripotency markers remained high for both cultures, confirming the maintenance of pluripotency.
The iPSC cells grown in 2 of the 8 culture conditions had reduced Tra1-- 60 and Tra1-- 81, however markers remained at starter culture levels in the other 6 conditions.

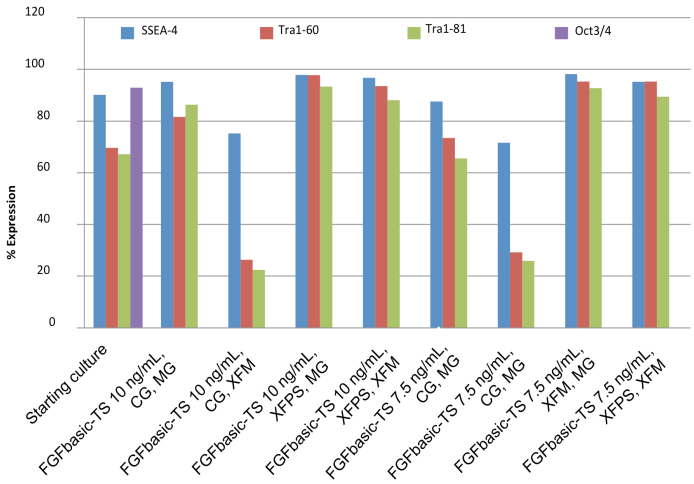


Figure3b. Pluripotency markers in iPSC cultures maintained with thermostable FGF basic, after 10 passages and for starter culture

Nanog Reporter Gene Analysis of Pluripotency

Nanog is a transcription factor required to maintain pluripotency that is often used as a pluripotency marker for stem cell cultures. Nanog expression of a hESC colony maintained using the 2-- day thermostable FGF basic feeding protocol was evaluated with a reporter gene assay using mCherry, a red fluorescent protein, see Figure 4. The undifferentiated cells in the hESC colony expressing Nanog produce a red color, while differentiated cells do not. Thermostable FGF basic maintained pluripotency in the hESC colony as shown by the red color of the colony.

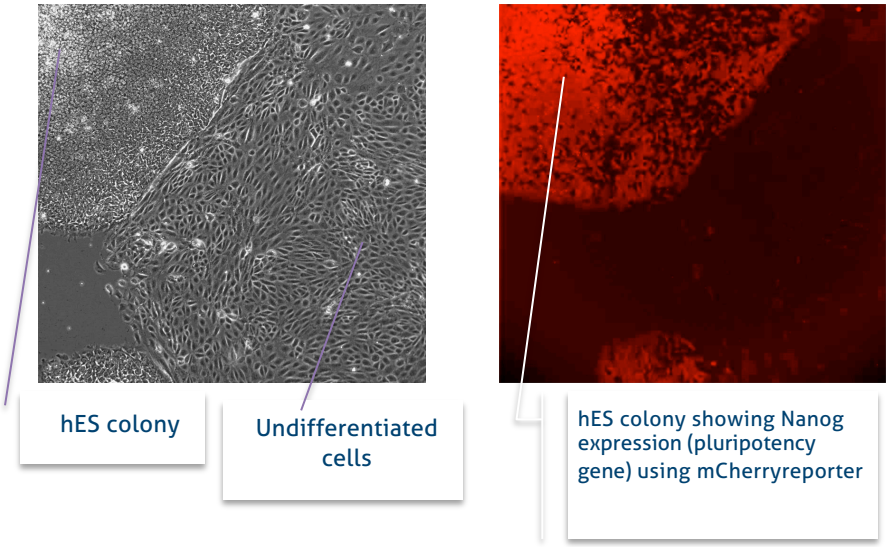
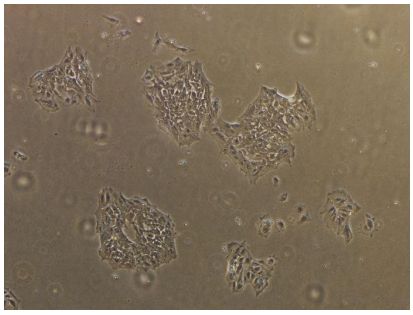


Figure 4. Nanog expression in a hES colony maintained with 2-- day feeding

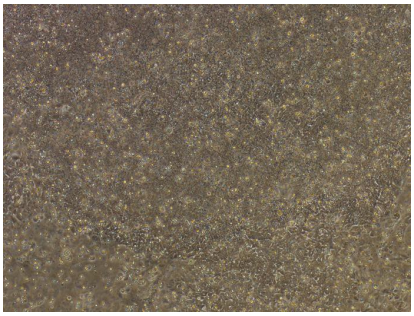
Cell growth characteristics

One of the functions of FGF basic is to promote the proliferation of stem cells. To confirm thermostable FGF basic maintains this biological function, cultures of iPSC cells grown with 2-- day feeding in including thermostable FGF basic at 7.5 ng/mL were evaluated for growth and proliferation characteristics. Culture conditions were animal-- free and xeno-- free media, matrix, and passage solution. As seen in Figure 5, iPSC cell cultures maintained healthy growth characteristics even after 10 passages, at day 2 and day 6.

Figure 5. iPSC culture @ 10th passage, 2-- day feeding protocol with thermostable FGF basic



Day 2

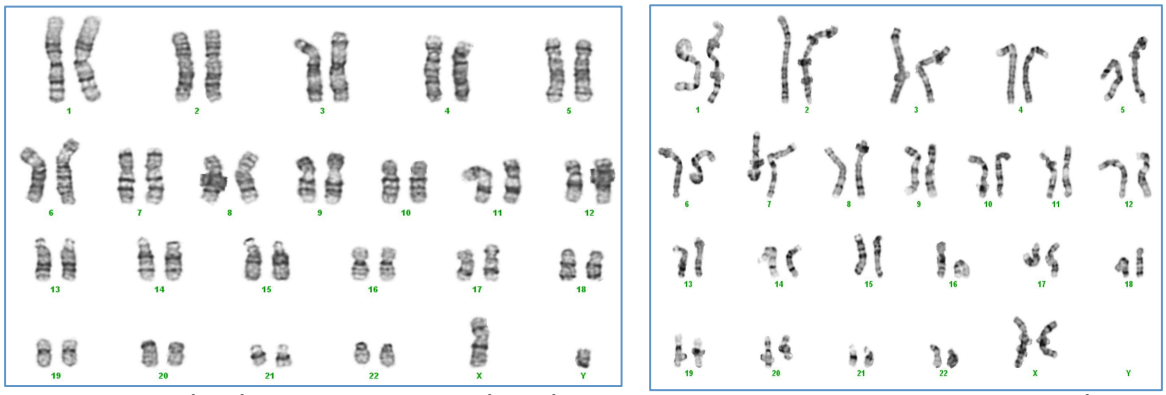


Day 6

Karyotyping

Stem cell cultures are known to develop genetic abnormalities during long-- term culture. Karyotyping analysis in an iPSC cell line was compared for starting cultures and for cultures after the 10th split, for selected best conditions. Both an hESC (data not shown) cell line and the iPSC cell line (shown in Figure 6) were cultured with 10 mg/mL thermostable FGF basic, xeno-- free matrix, and xeno-- free passaging solution.

Figure 6. Karyotype of hESC culture maintained with thermostable FGF basic on a 2-- day feeding schedule



Starter culture (left) and after 10 splits (right). The karyotype analysis for both cell lines (iPSC shown above) after the 10th split was normal.

Summary

Biochemical and cell culture analysis were performed to determine the stability and suitability of thermostable FGFbasic for a 2-- day feeding schedule. Thermostable FGF basic produced in HEK293 cells showed superior biochemical stability in cell culture media, and improved resistance towards proteolytic degradation by trypsin compared to FGF basic from *E. coli*. In cell culture in a xeno-- free, chemically defined cell culture system, both hESC and hiPSC cell lines with 10 ng/mL FGFbasic-- TS maintained robust levels of pluripotency markers (SSEA-- 4, Tra1-- 60, Tra1-- 81), normal karyotypes, and expression of Nanog after 10 passages.

These studies also demonstrate thermostable FGF basic can be used in a defined, xeno-- free, cGMP-- compatible cell culture system with a 2-- day feeding schedule to maintain good cell growth activity, and maintain pluripotency and differentiation potential in hESC and iPSC cell lines.