PRODUCT FOCUS

FGF Basic Thermostable

Maintains cell growth, pluripotency, and differentiation potential with a 2-day feeding schedule.

Catalog number:
HZ-1285

Activity:
0.05-0.4 ng/mL

Molecular Mass:
17 kDa, monomer, non-glycosylated

Species Reactivity:
Human, Mouse

HEK293 expressed

Animal component free

Endotoxin free | Tag free

- **Broad applicability for stem cell culture - can be used for multipotent or oligopotent cell types before they differentiate into mature effector cell types and also for Mesenchymal Stem Cells (MSCs), Neuronal Stem Cells (NSCs), and Hematopoietic Stem Cells (HSCs).**
- **Thermostable - maintains cell cultures more efficiently and homogeneously.**
- **Cost-effective - requires less frequent media changes for cell culture.**
- **High biological activity - can retain its biological activity for over 2-3 days at 37°c.**

FGF Basic TS Background

Basic fibroblast growth factor (FGF basic), also known as bFGF, FGF-2, FGF-B, or HBGF-2, belongs to the FGF family.

FGF plays important roles in diverse biological functions *in vivo* and *in vitro*.

FGF is involved in embryonic development, neuron differentiation, and the proliferation of cells of mesodermal origin and many cells of neuroectodermal, ectodermal, and endodermal origin[1,2].

Proteintech has developed a Thermostable FGF (FGF Basic TS)

FGF is a required component of stem cell culture media for maintaining cells in an undifferentiated state. Because FGF is unstable, daily media changes are needed.

Proteintech has developed a thermostable FGF basic (FGF basic TS) that supports a two day media change schedule, so no media changes are required over a weekend (Figure 1).

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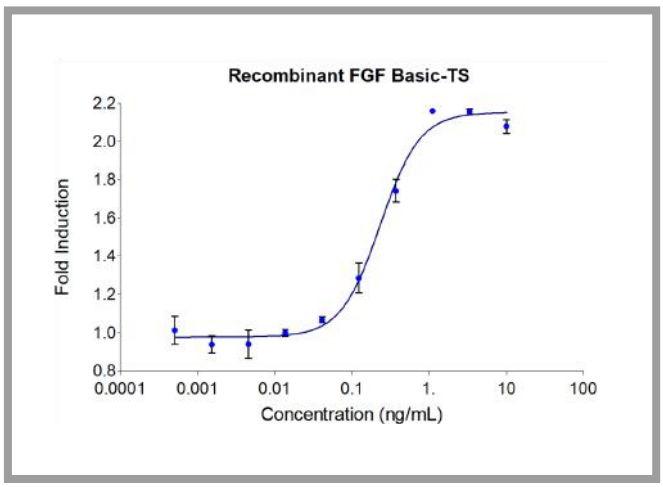

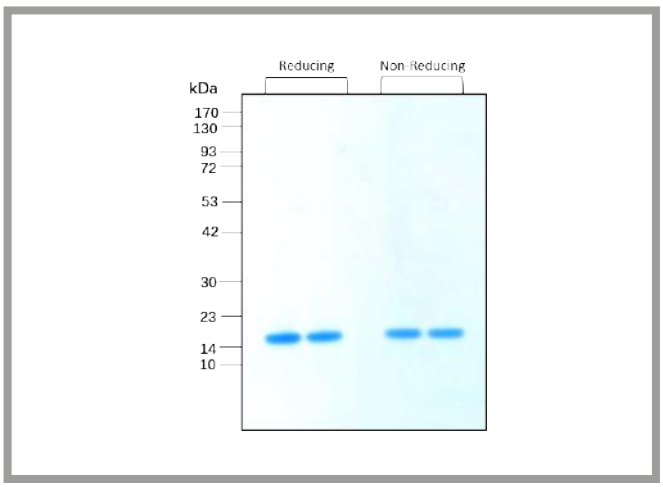

Figure 1. Left: The activity of FGF basic TS was determined by the dose-dependent stimulation of the proliferation of the Balb/c 3T3 cell line using Promega CellTiter96 Aqueous Non-Radioactive Cell Proliferation Assay.

Right: FGFbasic-TS was resolved by SDS-PAGE and stained with Coomassie blue. (R) represents reducing conditions and (NR) represents non-reducing conditions.

Proteintech FGF Basic TS is created in HEK293 cells using animal free components.

FGF basic is essential for stem cell culture[3]. Current offerings in the market are made using bacteria or animal cells, which raises the risk of pathogentransmission[4].

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 Proteintech's proprietary human cell line (HEK293) developed for the production of authentic human recombinant proteins.

FGF basic TS has greater stability than E. coli derived FGF basic, lasting 3x longer at 37˚C (Figure 2).

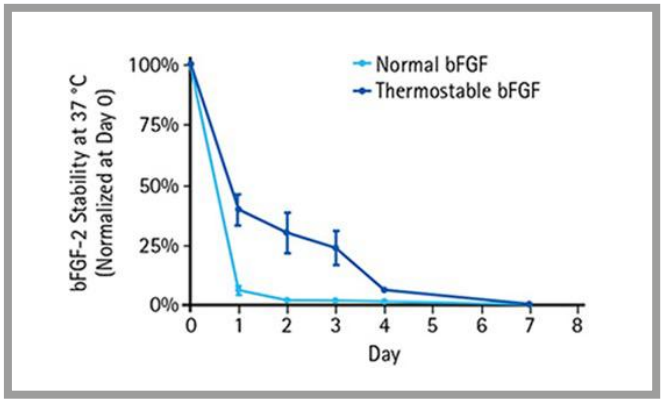

Figure 2. Enhanced stability of TS bFGF-basic at 37°c vs normal bFGF Source: HumanKine® Thermostable bFGF, an engineered recombinant protein with enhanced biological functionality on human iPS and neural progenitor cells.

By: Nick Asbrock, Christine Chen and Vi Chu, EMD Millipore, Bioscience Division, Temecula, CA, USA.*

Biochemical and cell culture analysis of FGF Basic TS.

Pluripotency Markers: SSEA-1, Tra 1-60, Tra1-81, Oct3/4

Molecular markers are one method to characterize the status of a pluripotent stem cell by their expression over passaging.

FGF basic TS was evaluated for effective maintenance of pluripotency markers using a two day feeding schedule in human embryonic stem cells (hESCs) and human-induced pluripotent stem cells (iPSCs).

Protocol Overview:

FGF basic TS was added at 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 passaged using either xeno-free, non-enzymatic passaging solution (XFPS) or a collagen-based reagent (CG).

Pluripotency cell surface markers were analyzed by flow cytometry for hESCand iPSC cultures grown in media containing 7.5 or 10 ng/mL FGF basic TS on the starting culture and after the 10th split, for all culture conditions (Figure 3 and 4).

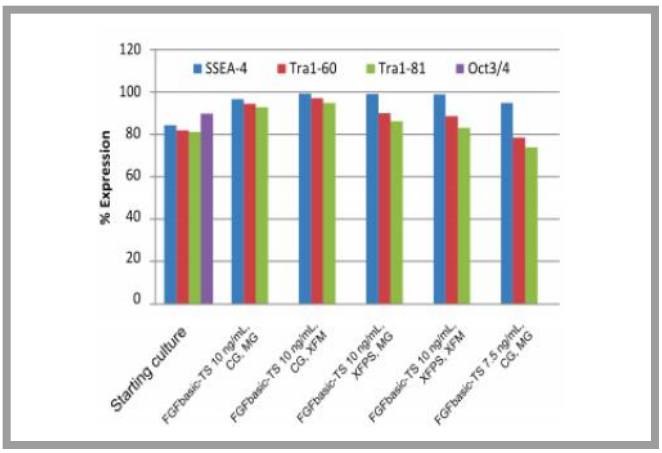

Figure 3. Pluripotency markers in hESC cultures maintained with thermostable FGF basic, in the starter culture and after 10 passages.

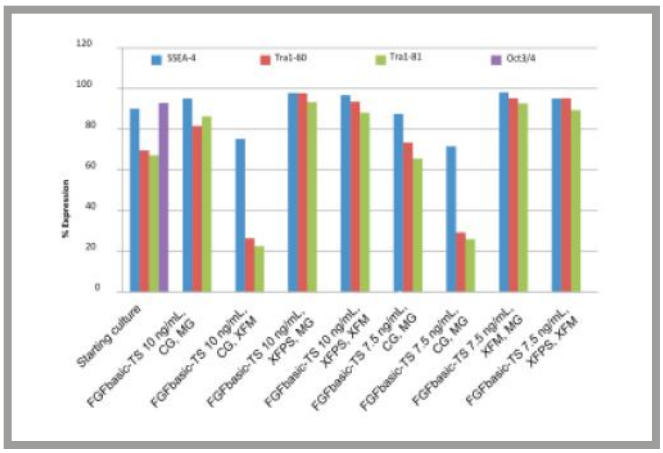

Figure 4. Pluripotency markers in iPSC cultures maintained with thermostable FGF basic, in the starter culture and after 10 passages.

Resistance to Enzymatic digestion

FGF basic and FGF basic TS were subjected to a tryptic digest then analyzed on an SDS-PAGE gel, without and with Heparin to stabilize against degradation by trypsin.

FGF basic was significantly degraded after 4.5 hours, while FGF basic TS protein levels were only slightly reduced (Figure 5).

FGF basic TS was more stable in cell culture media and more resistant to proteolytic degradation compared to FGF basic.

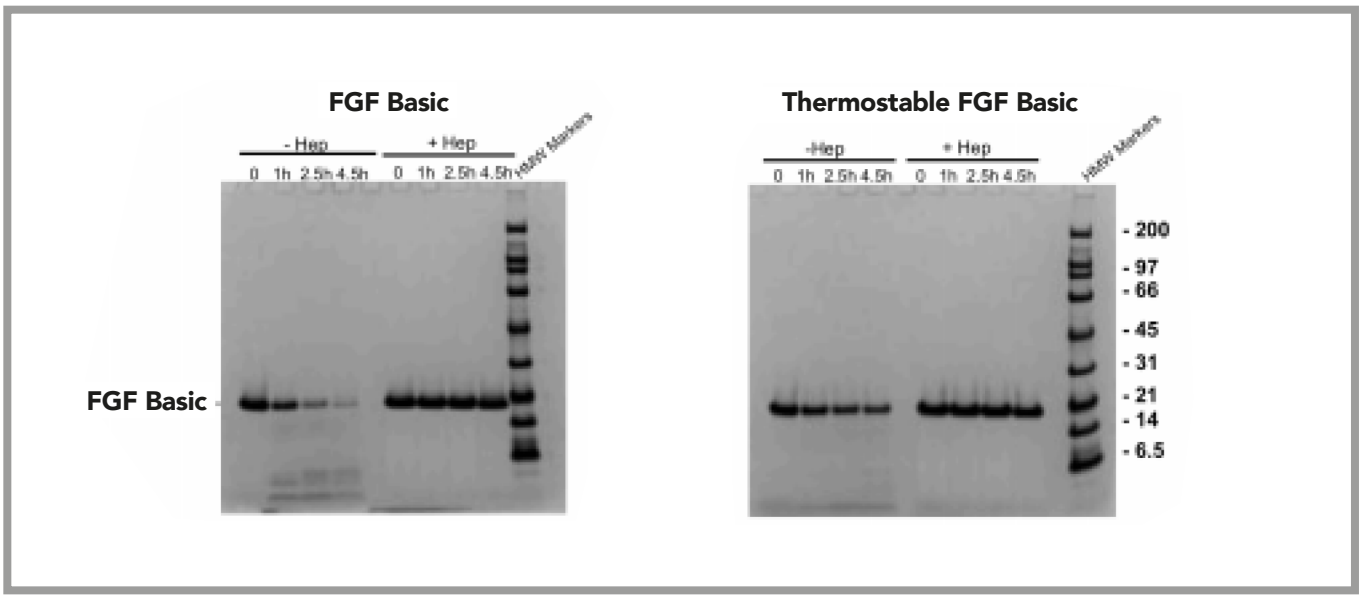

Figure 5. Trypsin digestion of FGF basic and FGF basic TS. HMW protein standard and FGF basic or FGF basic TS were loaded onto a 4-12% Nu-PAGE Bis-Tris gel. The gel was electrophoresed under non-reducing conditions.

References:

1. Stemple D, Mahanthappa N, Anderson D. Basic FGF induces neuronal differentiation, cell division, and NGF dependence in chromaffin cells: A sequence of events in sympathetic development. Neuron.
1988;1(6):517-525.

2. Rydel R, Greene L. Acidic and basic fibroblast growth factors promote stable neurite outgrowth and neuronal differentiation in cultures of PC12 cells. The Journal of Neuroscience.
1987;7(11):3639-3653.

3. Xu R, Peck R, Li D, Feng X, Ludwig T, Thomson J. Basic FGF and suppression of BMP signaling sustain undifferenti-ated proliferation of human ES cells. Nature Methods. 2005;2(3):185-190.

4. Pera M. Stem cell culture, one step at a time. Nature Methods. 2005;2(3):164-165 non-reducing conditions.