NEW PRODUCT

Stable Fibroblast Growth Factor 2

› For research applications

- › FGF2, also known as basic FGF, bFGF
- › Hyperstable protein
- › Thermal stability increased by 15°C compared to the wild-type
- › More than 5-times prolonged half-life in human cell culture incubated at 37°C
- › Engineered with fully retained biological function
- › No harmful stabilizing additives

Introduction

FGF2-STAB is a stabilized growth factor that offers a novel way to grow FGF2-dependent cell cultures more efficiently, with fewer media changes. FGF2-STAB retains full biological activity even after five days at 37°C. The stable level of FGF2 in culture allows for a more homogenous, undifferentiated stem cell culture, while saving researchers valuable time and money, as repeated supplementation by FGF2 and every day medium change is not required.

FGF2 is a non-glycosylated heparin binding growth factor that is expressed in the brain, pituitary gland, kidney, retina, bone, testis, adrenal gland, liver, monocytes, epithelial cells and endothelial cells. FGF2 functions as a pleiotropic regulator of proliferation, differentiation, migration, and survival in a variety of cell types and is an essential component of media for the cultivation of human pluripotent stem cells because it helps maintain the cells in the pluripotent state. This property makes cells valuable for studying embryogenesis, for drug discovery, and for cell-based therapies.

STABLE FIBROBLAST GROWTH FACTOR 2

STABLE FIBROBLAST GROWTH FACTOR 2 CAT. NO.: RENT001010 SIZE: 0.01 mg CAT. NO.: RENT001050 SIZE: 0.05 mg CAT. NO.: RENT0011000 SIZE: 1 mg	
Type	Recombinant human protein, *E. coli*-derived
Purity	>95% by SDS PAGE under reducing conditions
Endotoxin	Endotoxin level is <0.1 ng/µg of protein (<1 EU/µg)
Formulation	Lyophilized from a filtered solution in 20 mM potassium phosphate buffer and 750 mM sodium chloride, pH 7.5
Storage & Stability	Upon arrival store at -20°C to -80°C. Lyophilized FGF2-STAB is stable for up to 12 months when stored at -20°C to -80°C.
Intended use	For research use only

Potential industrial applications

FGF2 is a pleiotropic regulator of proliferation, differentiation, migration, and survival in a variety of cell types and is an essential component of media for the cultivation of pluripotent stem cells because it helps maintain the cells in the pluripotent state [1, 11-14]. This property makes cells valuable for studying embryogenesis, for drug discovery, and for cell-based therapies.

Research shows that FGF2 plays an important role in wound healing [2], diabetic foot ulcer treatment [3], periodontal regeneration [4], bone regrowth [5], cancer treatment [6], cardioprotection [7], neuroprotection [8], and treatment of mood disorders [9]. A key driver in these fields is the aging of the global population, since age-related diseases increase a risk of damage to tissues and slow the healing process.

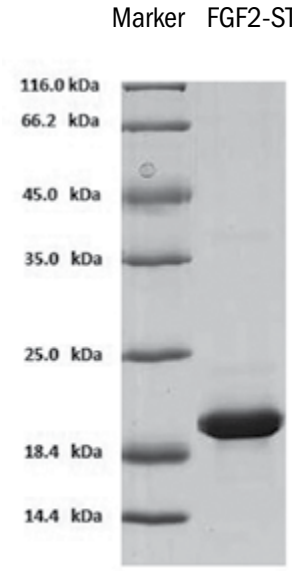
FGF2 has been shown to increase skin elasticity, decrease the depth of wrinkles, increase hydration of skin, decrease the depth of pigmentation and promote hair growth by inducing anagen phase of hair follicles [10, 15, 16]. Potential applications to skin care include rejuvenation of the epidermal cells and the underlying fibroblast cells, which produce collagen, elastin and hyaluronic acid.

Please request additional information before use in applications that require regulatory approval.

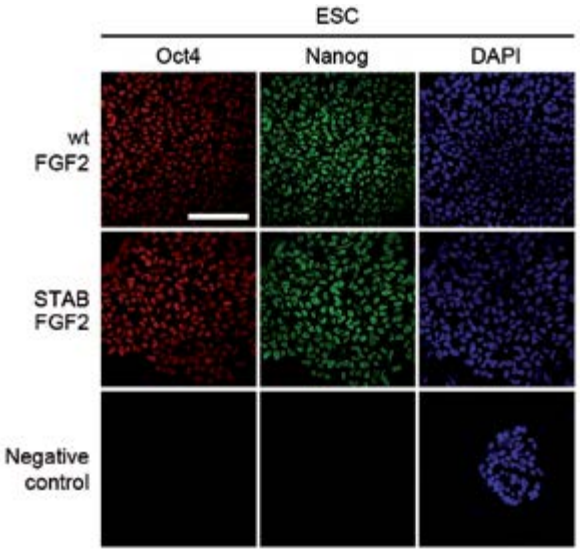
› International patent pending

STABLE FIBROBLAST GROWTH FACTOR 2

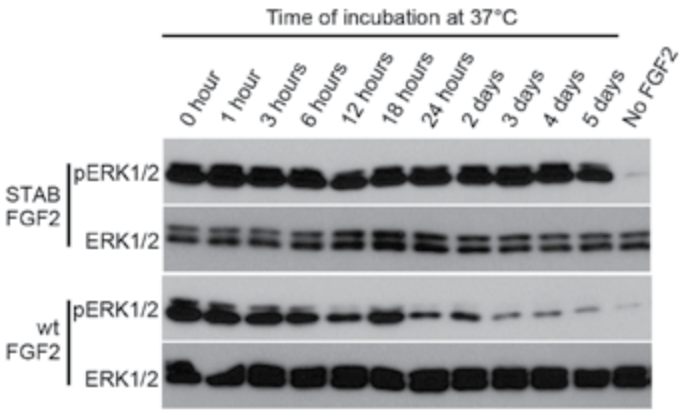
Application Data



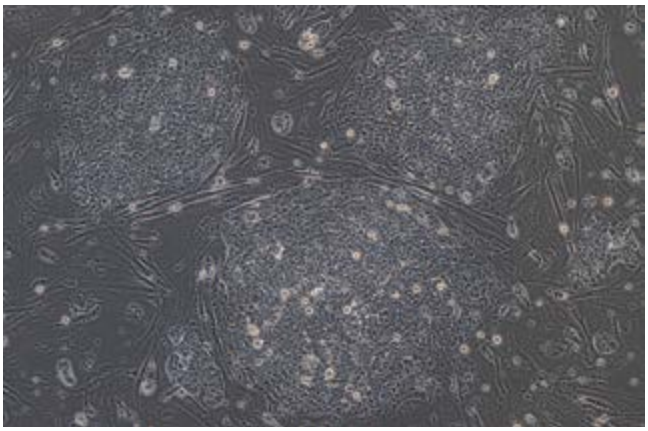
A) Image of SDS-PAGE gel showing purity of isolated FGF2-STAB.
Protein marker: 116, 66, 45, 35, 25, 18, 14 kDa.



B) FGF2-STAB maintains pluripotency marker expression of human embryonic stem cells (ESC) equally with the wild-type. After five passages, cells were immunostained for pluripotency markers Oct4 and Nanog. Negative controls were incubated without antibodies. Scale bars, 100 µm.



C) Medium was supplemented with 10 ng/ml FGF2 and incubated at 37°C for 1 hour-5 days. Then, FGF2-starved human ESC were treated with pre-heated medium and immuno- blotted for phosphorylated ERK1/2 kinase. While the biological activity of the wild-type declined with time of heat-preincubation, the stabilized FGF2-STAB retained full biological activity even after five days at 37°C.



D) FGF2-STAB maintains undifferentiated morphology of human pluripotent stem cells. Human ESC (CCTL14) were propagated as typical tightly packed colonies in the presence of mouse embryonic fibroblast feeder layer. The culture medium was supplemented by 4 ng/ml of FGF2-STAB.

STABLE FIBROBLAST GROWTH FACTOR 2

Proliferation of NIH/3T3 cells by FGF2-STAB

Test principle

Human embryonic stem cell cultures need FGF2 in the media to remain in an undifferentiated and pluripotent state. Since FGF2 is highly unstable (its functional half-life is only 9 h at 37°C), daily media changes with fresh FGF2 are required, which can be both time- and money-consuming. Enantis has developed a stable variant of 155-amino-acid human FGF2 (**FGF2-STAB**), exhibiting a half-life increase of 25-fold compared to the wild-type. This unprecedented stabilization was achieved using a computer-assisted protein engineering strategy for designing new proteins with new or improved properties.

In this study, a proliferation assay using NIH/3T3 cells was performed in order to demonstrate that FGF2-STAB can maintain good cell growth and pluripotency. Comparison of FGF2-STAB with human FGF2 wild-type revealed that after 48-hour incubation at 37°C the wild-type has lower capacity to promote 3T3 cell proliferation than engineered FGF2-STAB. FGF2-STAB exhibited an ED50 as much as 5-fold lower than the wild-type protein, demonstrating that FGF2-STAB has increased thermal stability.

Materials and methods

FGF2 VARIANTS

FGF2-STAB was engineered for higher stability, but still shares more than 92% amino acid sequence identity with the 155-amino-acid human FGF2; having the following sequence: MAAGSITTLPA LPEDGGSGAF PPGHFKDPKR LYCKNGGFFL RIHPDGRVDG VREKSDPHIK LQLQAEERGV VSIKGVCANR YLAMKEDGRL LASKCVTDEC FFFERLESNN YNTYRSRKYT SWYVALKRTG QYKLGSKTGP GQKAILFLPM SAKS. Both FGF2-STAB and FGF2 wild-type were produced recombinantly in *E. coli* as His-tagged proteins and purified by affinity chromatography (purity > 95% according to SDS-PAGE).

PROLIFERATION ASSAY

NIH/3T3 cells were seeded in a density of 40,000 cells/cm^2 in 190 µl of medium per well (DMEM 31966, Gibco® + P/S + 10 % newborn calf serum). After 24 hours, media was changed for starvation (DMEM 31966, Gibco® + P/S + 0.5 % newborn calf serum). After 16 hours, FGF2-STAB or FGF2 wild-type were diluted in sterile water to final concentrations of 0.01 – 20 ng/ml and added to the cells, which were cultured for an additional 48 hours at 37 °C. Cell proliferation was measured using CyQuant® fluorescence assay.

Results

The ED50 for FGF2-STAB, i.e., the concentration of FGF2-STAB that produces one-half the maximal response, as determined in a proliferation assay of NIH/3T3 cells, is 0.6-1.1 ng/ml.

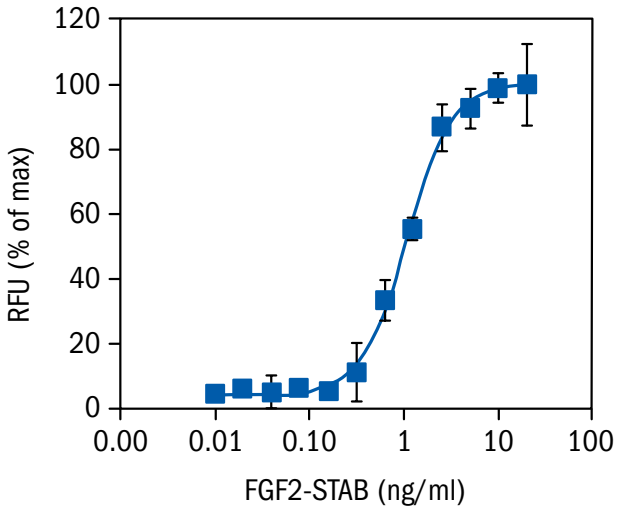


Figure 1: *Proliferation of NIH/3T3 cells induced by FGF2-STAB recombinant protein.*

The bioactivities of recombinant FGF2-STAB and the wild-type FGF2, as determined in a NIH/3T3 proliferation assay, were compared. The ED50 value of FGF2-STAB was about 5-times lower than that of the wild-type. In other words, under the same experimental conditions, less FGF2-STAB is needed to achieve a given effect on cell proliferation. This may be explained by the fact that FGF2-STAB has higher stability and exhibits a prolonged half-life at 37°C compared to the wild-type (cells were treated for 48 hours at 37°C).

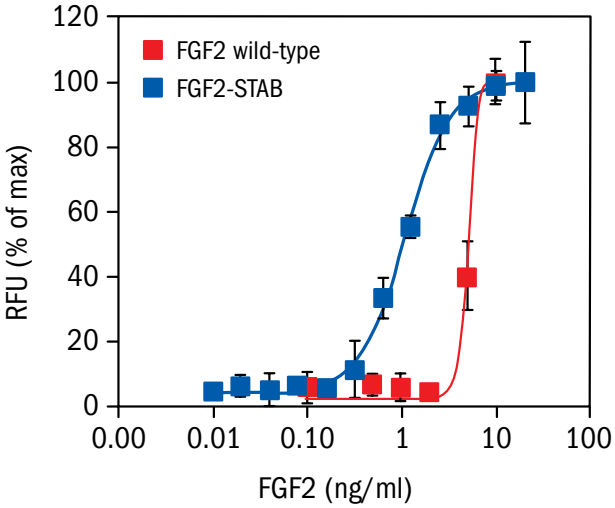


Figure 2: *Proliferation of NIH/3T3 cells induced by the wild-type FGF2 (red) and engineered FGF2-STAB (blue).*

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Enantis s.r.o.
Kamenice 34, 625 00 Brno, Czech Republic
Phone: +420 549 495 178
E-mail: enantis@enantis.com

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BioVendor
Research
and Diagnostic Products

BioVendor – Laboratorni medicina a.s.
Karasek 1767/1, 621 00 Brno, Czech Republic
Phone: +420 549 124 185, Fax: +420 549 211 460
E-mail: info@biovendor.com

BioVendor GmbH
Otto-Hahn-Straße 16, 34123 Kassel, Germany
Phone: +49 6221 4339 100, Fax: +49 6221 4339 111
E-mail: infoEU@biovendor.com

BioVendor GesmbH
Nußdorfer Straße 20/10, 1090 Vienna, Austria
Phone: +43 1 890 9025, Fax: +43 1 890 9025-15
E-mail: infoAustria@biovendor.com

BioVendor, LLC
128 Bingham Rd., Suite 1300, Asheville, NC 28806, USA
Phone: +1-800-404-7807, Phone: +1-828-575-9250
Fax: +1-828-575-9251, E-mail: infoUSA@biovendor.com

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