

Enantis

Stable Fibroblast Growth Factor 2 FGF2-STAB[®]

For research applications

- **FGF2, also known as basic FGF, bFGF**
- **Hyperstable protein**
- **More than 20-times the standard 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 twenty 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 because repeated supplementation of FGF2 and a daily 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.

FGF2-STAB®

CAT. NO.: FGF2STAB00010

SIZE: 0.01 mg

CAT. NO.: FGF2STAB00050

SIZE: 0.05 mg

CAT. NO.: FGF2STAB01000

SIZE: 1 mg

Type	Recombinant human protein, <i>E. coli</i> -derived, His-tagged
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]. Wild-type FGF2 is highly labile at 37°C (its half-life is as low as 9 hours), therefore, daily media changes with fresh FGF2 are needed and cells are exposed to fluctuation of FGF2 concentration. FGF2-STAB® stable biological activity means fewer media changes and no fluctuation in concentration.

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. A prolonged and sustained delivery of FGF2-STAB® provides greater therapeutic effect and faster healing.

FGF2 has been shown to increase skin elasticity, decrease the depth of wrinkles, increase the hydration of skin, decrease the depth of pigmentation and promote hair growth by inducing the 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. Since it is extremely difficult to keep labile FGF2 active in cosmetic products, stable FGF2-STAB® could truly make a difference.

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

International patent pending

Application Data

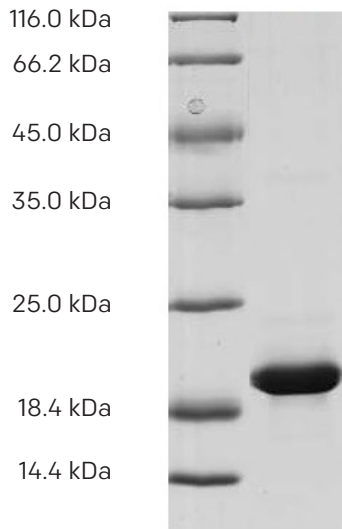
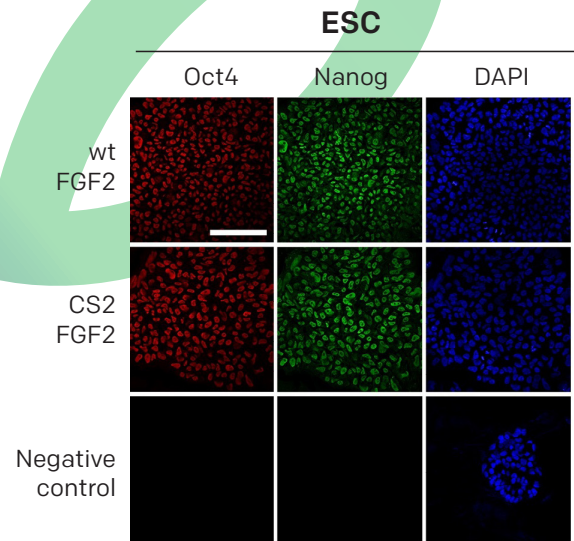
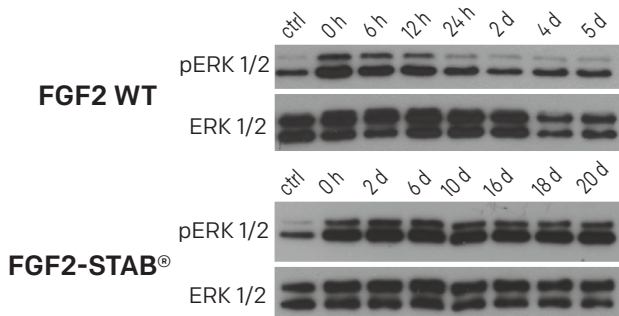


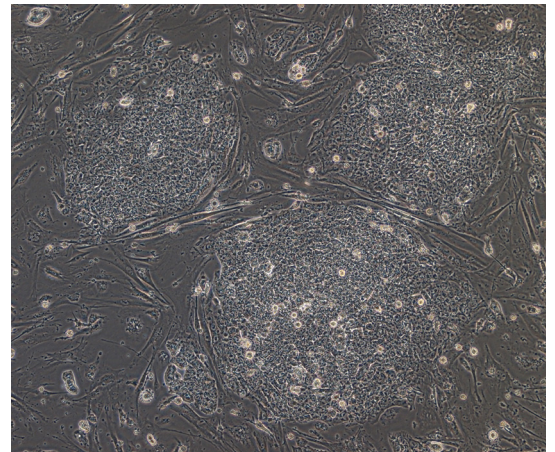
Image of SDS-PAGE gel showing purity of isolated FGF2-STAB[®]. Protein marker: 116, 66, 45, 35, 25, 18, 14 kDa.



FGF2-STAB[®] maintains the 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 μ m.



Medium was supplemented with 10 ng/ml FGF2 and incubated at 37°C for 6, 12 and 24 hours, and 2, 6, 10, 16, 18, and 20 days. Then, FGF2-starved hPSC were treated with CM containing pre-incubated FGF2 for two hours and Western blotted for phosphorylated ERK1/2. While the biological activity of the wild-type declined with time of heat-preincubation, the stabilized FGF2-STAB[®] retained full biological activity even after twenty days at 37°C.



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

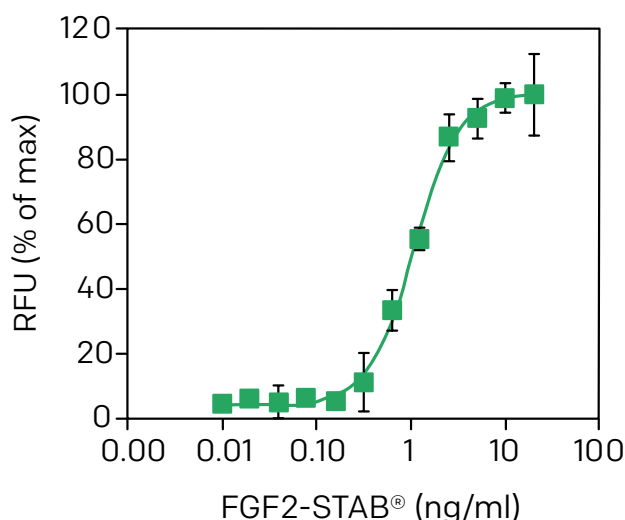
Proliferation of NIH/3T3 cells by FGF2-STAB®

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.

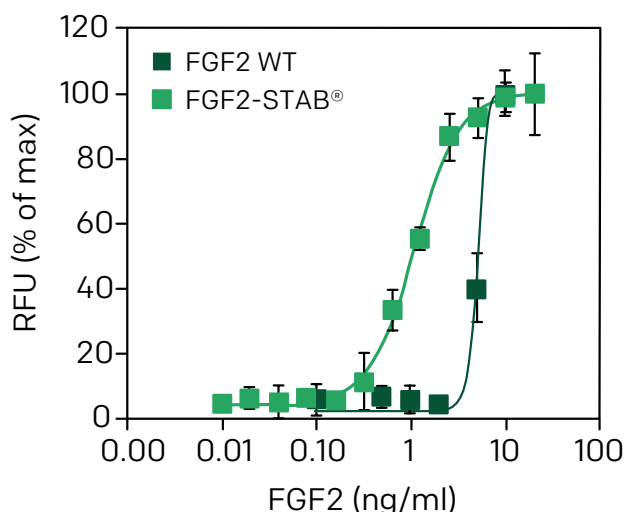
In this study, a proliferation assay using NIH/3T3 cells was performed in order to demonstrate that FGF2-STAB® can maintain stable cell growth and pluripotency. NIH/3T3 cells were seeded in a density of 40,000 cells/cm² in 190 µl of medium per well (DMEM 31966, Gibco® + P/S + 10 % newborn calf serum). After 24 hours, the 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



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. 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.



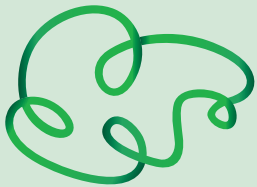
The bioactivities of recombinant FGF2-STAB® and the wild-type FGF2, as determined in a NIH/3T3 proliferation assay, were compared. The ED50 value of FGF2-STAB® was about 5-times lower than that of the wild-type. In other words, under the same experimental conditions, less FGF2-STAB® is needed to achieve 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 (dark green) and engineered FGF2-STAB® (green).

References

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Enantis s.r.o.

Supporting innovative biotechnologies by engineering improved proteins to create a healthier and more sustainable future.

Our expertise is based on vast experience in protein science and state-of-the-art software tools. A combination which enables us to provide powerful and unique protein optimization services to our clients.

We're also responsible for the production and sale of novel proteins. Our R&D activities are predominantly focused on the family of fibroblast growth factors (FGFs). The goal is to improve the poor stability of FGFs for therapeutic use. Where FGF2 is concerned, we are already in the preclinical testing phase.

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