FGF-2 STAB®

Scientific Support Document

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Introduction

Product description:

FGF-2, also known as basicFGF, is a growth factor that controls fundamental biological processes and is qualified for different applications including industrial cell-based meat production, cell culture and cell therapy manufacturing.

Core Biogenesis has licensed from Enantis a thermostable (heat stable) FGF-2 called FGF-2 STAB ® (FGF-2-G3). We use our proprietary manufacturing platform to produce FGF-2 STAB, our production process is animal-free, endotoxin-free and biorisk-free, with unparalleled scalability and cost-reduction possibilities.

FGF-2-STAB® is a stabilized growth factor that offers a novel way to grow FGF-2-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 FGF-2 in culture allows for a more homogenous, undifferentiated stem cell culture, while saving researchers valuable time and money because repeated supplementation with FGF-2 and a daily medium change is not required.

Product Overview

Core Biogenesis FGF-2-STAB® is produced by recombinant expression of the human sequence of FGF-2 in plant seeds of *Camelina sativa.* The general sequence for FGF-2 is available at: https://www.uniprot.org/uniprot/P09038. Modifications to the sequence for improved expression, bioactivity and stability are applied but are proprietary.

None of the components or raw materials employed for the production of FGF-2-STAB® are derived or extract from animal or human origin.

Specifications:

Criteria	Results
Purity	≥ 95% measured by SDS page.
Bioactivity	EC50 < 1 ng/ml.
Endotoxin levels	≤ 0.005ng/μg (≤ 0.005EU/ug of protein).
Animal/Human components	Free
Formulation	PBS pH 7.4

Fig.1 Results on product features in relation to quality control analysis determined for purity, bioactivity, endotoxin levels, initial formulation and animal/human derivatives.

Manufacturing and Control

Quality Standards:

Core Biogenesis is currently in the process of establishing a GMP compliant production facility to produce biorisk-free cGMP growth factors and cytokines in our facility in Strasbourg, France. Our current biorisk-free products nonetheless represent a significant improvement in safety over products produced using traditional production platforms based on bioreactors and other expression hosts.

Prior to filling of the sterile container, FGF-2-STAB® is manufactured in liquid form. It is then filtered at 0.2 µm following aseptic technique and following standard operation procedures.

Disclaimer: This product is not intended for any therapeutic or diagnostic use in humans or animals. Do not use internally or externally in humans or animals. For Research Use Only.

Data & Performance

Product bioactivity measured by proliferation assay of NIH/3T3 cells with FGF-2 STAB®:

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. This product is 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/cm^2 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.

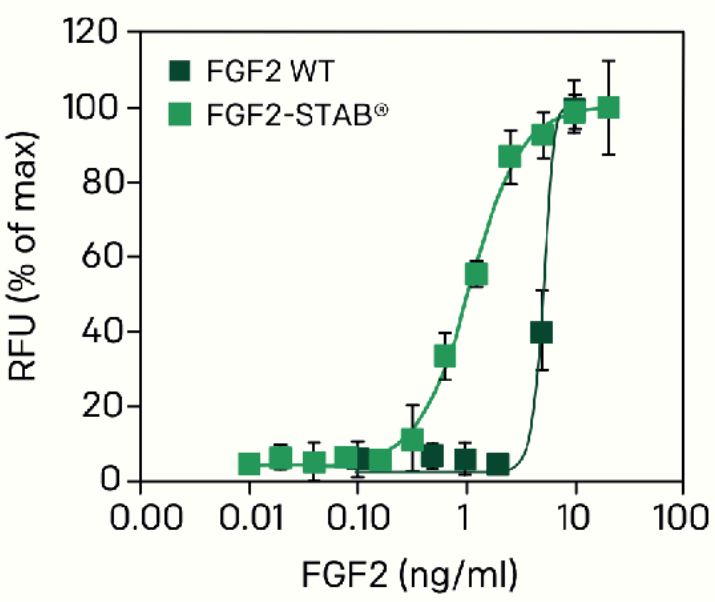

Fig. 2 Comparison of FGF2-STAB® with human FGF-2 wild-type revealed that after 48-hour incubation at 37°C the wild-type has lower capacity to promote 3T3 cell proliferation than engineered FGF2-STAB®. FGF2-STAB® exhibited an ED50 as much as 5-fold lower than the wild-type protein, demonstrating that 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.

Additional data and figures

Sourced from Enantis s.r.o.

Product stability:

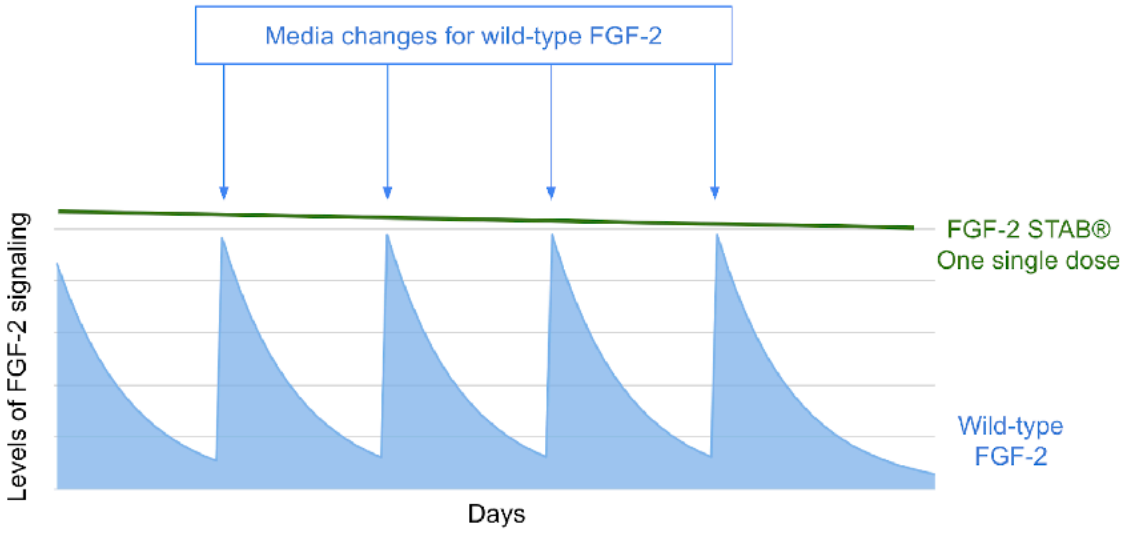

Fig. 3 The ability of FGF-2 variants (WT and STAB) were tested to induce the proliferation of NIH/3T3 cells under serum-free basal media at 10ng.mL^{-1}. Cell were pre-incubated at 37°C for 6 h, 12 h, 24 h, 48 h and 72 h in triplicates for each condition. At each time point, cells were collected and lysates were Western blotted for phosphorylated ERK1/2. Blots per each protein variant were analyzed and band densities were plotted as a function of pre-incubation time.

Product purity and signalling levels:

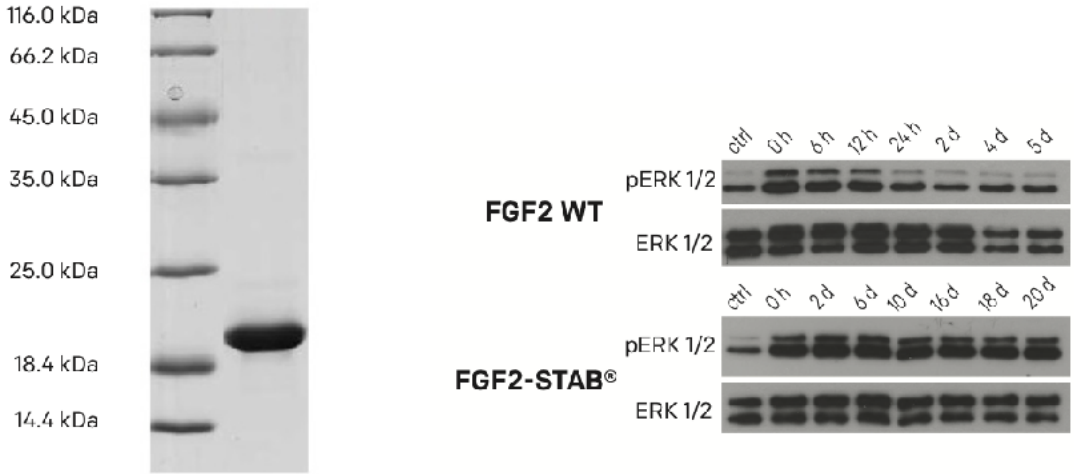

Fig. 4 (A) Image of SDS-PAGE gel showing purity of isolated FGF2-STAB®. Protein marker: 116, 66, 45, 35, 25, 18, 14 kDa. (B) Medium was supplemented with 10 ng/ml FGF2 and incubated at 37°C for 6, 12 and 24 hours, and 2, 6, 10, 16, 18, and 20 days. Then, FGF2-starved hPSC were treated with CM containing 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.

Sourced from Enantis s.r.o.

CORE BIOGENESIS

In partnership with

SCIENTIFIC LABORATORY SUPPLIES

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Product performance with pluripotent cells hESCs:

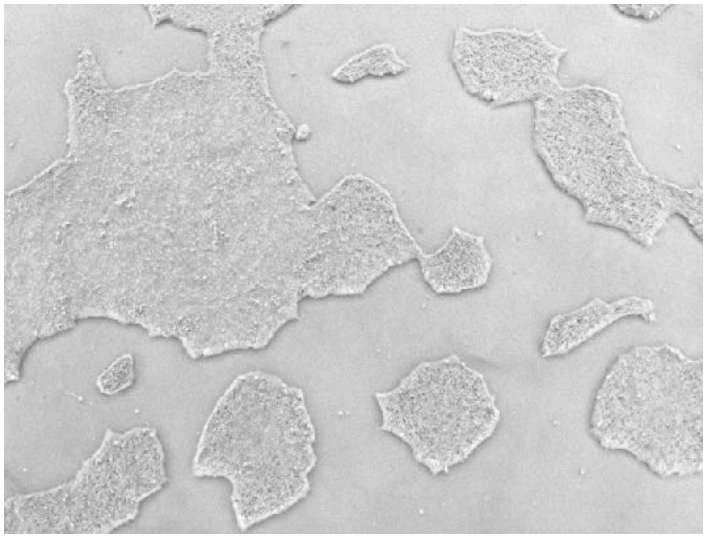

Fig. 5 FGF2-STAB® maintains an undifferentiated morphology of human pluripotent stem cells. Human ESC (CCTL14) were propagated as typical tightly packed colonies.
The culture medium was supplemented 10 ng/ ml of FGF2-STAB®.

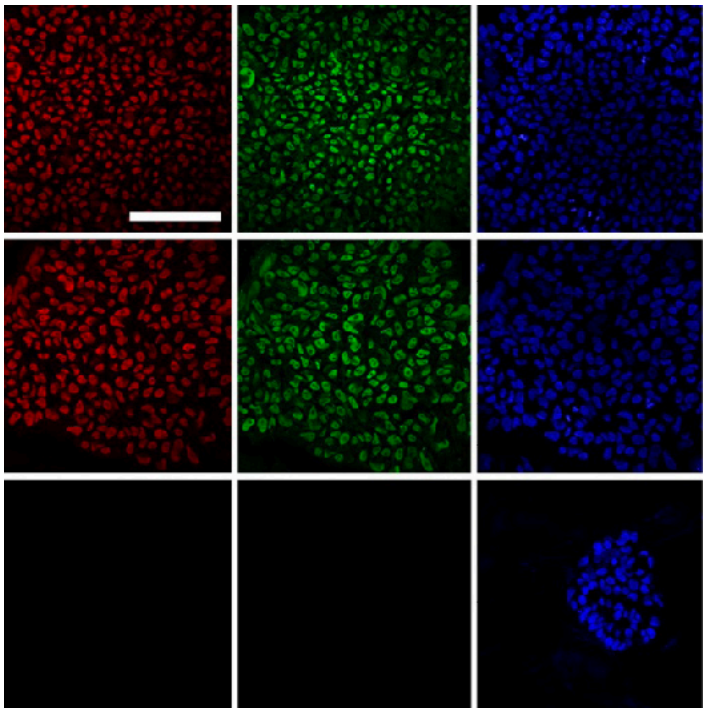

Fig. 6 FGF2-STAB® maintains the pluripotency marker expression of human embryonic stem cells (ESC) equally with the wild-type. After five passages, cells were immunostained for pluripotency markers Oct4 and Nanog. Negative controls were incubated without antibodies. Scale bars, 100 µm.

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