



FGF-2 PURE

Scientific Support Document

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Introduction

Product description:

FGF-2, also known as basicFGF, is a growth factor that is produced mainly in fibroblasts, endothelial cells and smooth muscle cells. FGF-2 controls fundamental biological processes of ectodermal, mesodermal and endodermal lineages including cell growth and differentiation, tissue formation, angiogenesis and wound healing. Recombinant human FGF-2 consists of 146 amino acids, 16.06 kDa.

Human FGF-2 can be used in a wide variety of areas for cell and molecular biology. We provide high purity and biocative protein with outstanding performance in: – Culture and long-term expansion of pluripotent stem cells (hESCs and iPSCs). – Growth stimulation for endothelial cells and mesenchymal stem cells (MSCs). – Neural differentiation (from PSCs), and Chondrogenic differentiation (from MSCs). – Culture of Bovine Satellite Cells (BSCs) and proliferating myoblasts.



Product Overview

Core Biogenesis purified recombinant human FGF-2 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 might be applied but are proprietary.

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

Specifications:

Criteria	Results
Purity	≥ 95% measured by SDS page.
Bioactivity	EC50 < 0.1 ng/ml.
Endotoxin levels	≤ 0.005ng/μg (≤ 0.005EU/ug of protein).
Animal/Human components	Free
Formulation	Tris pH 7.4. Shipped lyophilised.

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, purified FGF-2 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 purified recombinant human FGF-2 :

Purified recombinant human FGF-2 (Core Biogenesis) is supplied in lyophilized format ready to use after reconstitution. To demonstrate the performance of the product as intended and in workflows where the FGF-2 may be stored as a diluted stock solution, the product was prepared as follows:

- An aliquot of purified recombinant human FGF-2 was dispensed into a sterile tube and diluted with DPBS/1%HSA to a final concentration of 0.1mg/mL. This solution was stored at -20°C prior to use.

Diluted purified FGF-2 (Core Biogenesis) was added to basal media (DMEM 31966, Gibco® + P/S + 10 %) and 3T3/NIH cells were cultured with serial concentrations of FGF-2 from 0.0001 ng/mL to 100 ng/mL. Each of the condition was replicated in triplicates. Briefly, 40,000 cells/cm² were cultured in 200 µl of medium per well and incubated at 37°C and 5% CO₂ for 20 hours. Cell proliferation was measured using CyQuant® XTT cell viability assay.

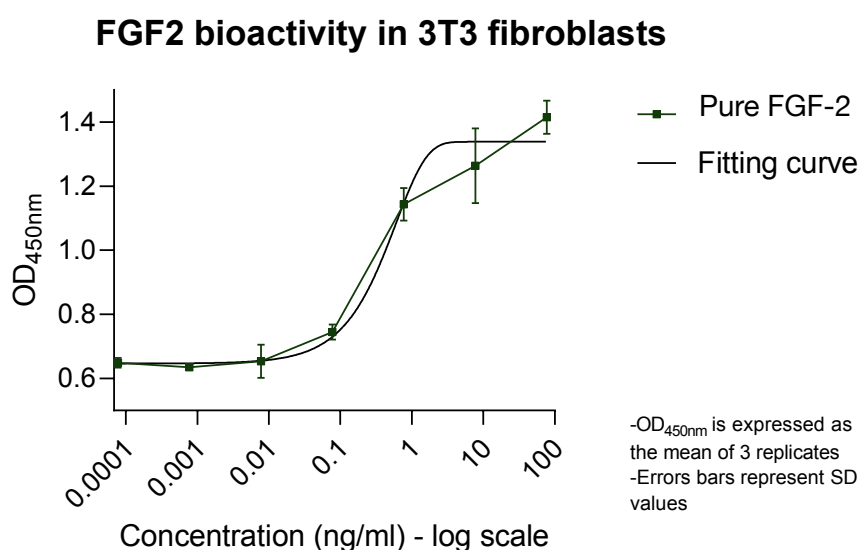


Fig. 2 Bioactivity assay of purified human recombinant FGF-2 measured by cell proliferation of 3T3/NIH cells. The EC50 value for this effect is 0.1 - 0.8 ng/mL.

CORE BIOGENESIS

In partnership with



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Additional data and figures

Product purity and signalling levels:

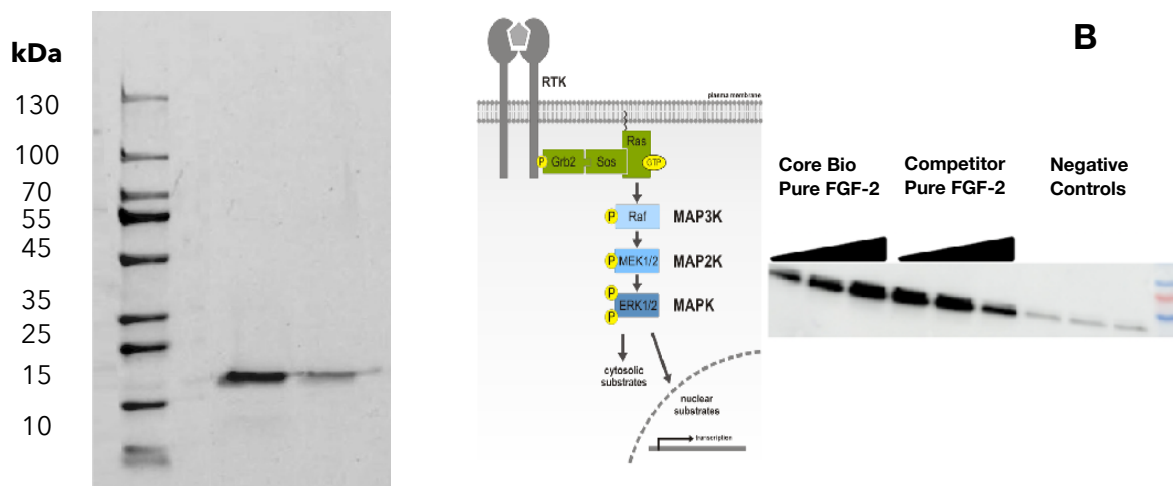


Fig. 4 (A) Image of SDS-PAGE gel showing purity of isolated purified human recombinant FGF2. Protein marker: 130, 100, 70, 55, 45, 35, 25, 15,10 kDa. (B) Medium was supplemented with 20 ng/ml of Core Biogenesis purified human recombinant FGF-2 or competitor human recombinant FGF-2, and incubated at 37°C for 6, 12 and 24 hours. Then, FGF2-starved 3T3/NIH cells were treated with media containing pre-incubated FGF2 for two hours and Western blotted for phosphorylated ERK1/2.

Product performance with pluripotent cells hESCs:

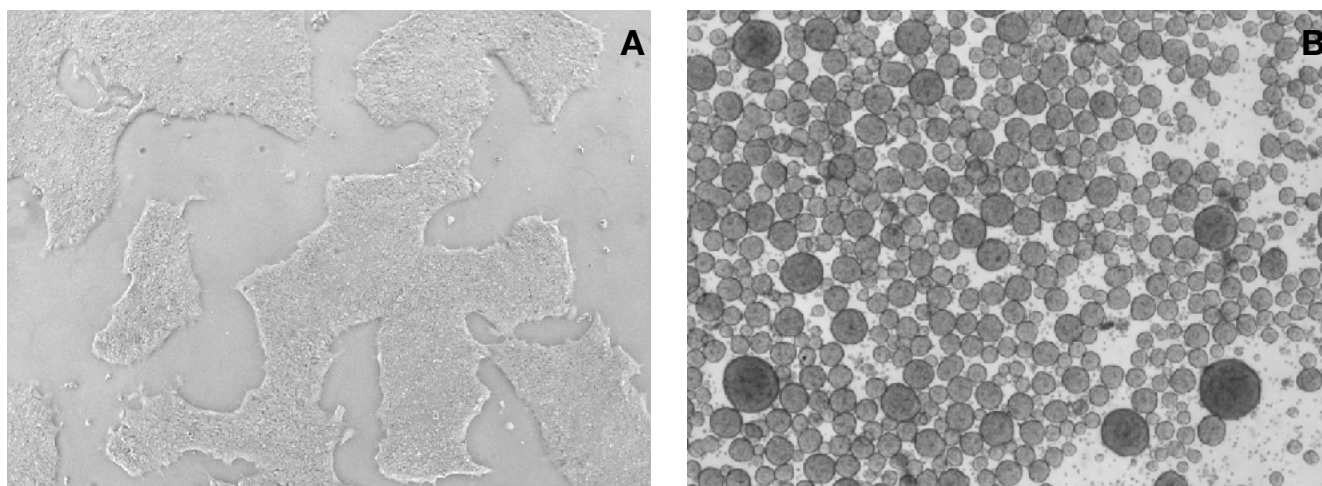


Fig. 5 Purified human recombinant FGF-2 maintains an undifferentiated morphology of human pluripotent stem cells. Human ESC (H1) were propagated in cell media based on E8 formulation as typical tightly packed colonies under feeder-free conditions in adherent culture (A) and created rounded embryoid bodies in 3D culture (B) using rocking platform shakers to promote the formation of homogeneous spheres. The culture medium in both cases was supplemented with 20 ng/ ml of purified human recombinant FGF-2 (Core Biogenesis).