FGF10-STAB® Polypeptide

Enantis

Stabilized Fibroblast Growth Factor 10

Hair growth-promoting ingredient with superior stability when compared to the native form of FGF10 polypeptide:

- ✓ **Enhances hair follicle activity**
- ✓ **Elongates anagen phase of hair cycle**
- ✓ **Activates keratinocytes**
- ✓ **Reduces microinflammation that causes hair loss**

Suitable for hair with thinning, non-growing hair, and an unhealthy scalp.

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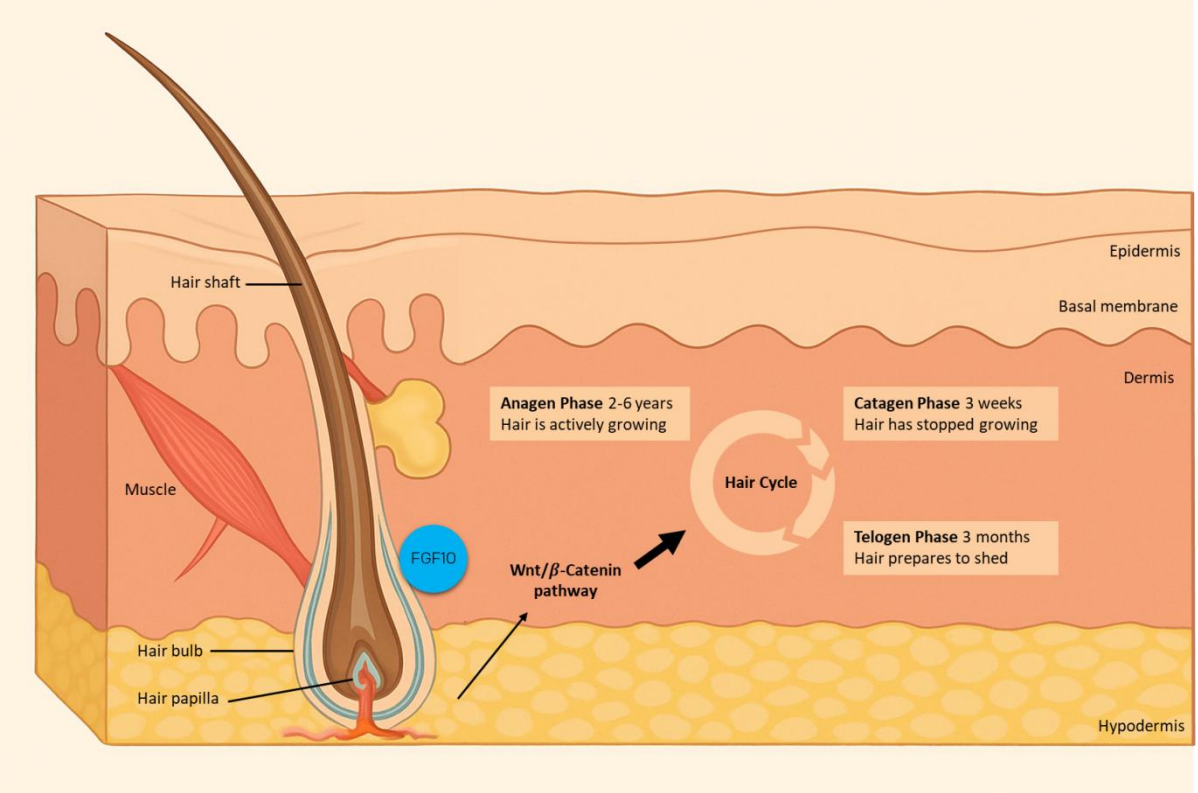
Fibroblast growth factor 10 (FGF10)

Fibroblast Growth Factor 10 (FGF10), also known as Keratinocyte growth factor 2 (KGF 2) is an important signalling molecule for many cellular processes in humans. It regulates **proliferation**, **differentiation, and migration** of various cell types [1]. As an example, it promotes re-epithelization due to stimulation of keratinocytes proliferation and migration [2]. FGF10 has also shown potential to accelerate wound healing in both *in vitro* and *in vivo* studies [3].

In addition to its **essential role during organogenesis** and **lung branching morphogenesis**, FGF10 is **involved in the processes of hair growth by hair-follicle cell proliferation** [1,4,5]. Three main phases of the hair growth cycle are visualised in the figure below as follows: anagen (growth phase), catagen (regression phase) and telogen (resting phase). The entire hair follicle cycle is regulated by the Wnt/β-catenin signalling pathway. An earlier transition from the telogen to the anagen phase (and its prolongation) is crucial for promoting hair growth. This transition can be induced by protein β-Catenin located in dermal papilla, as well as by secreted signalling molecule Sonic hedgehog (Shh) [4-6].



FGF10 is found in dermal papilla fibroblasts and is binding to the fibroblast receptor 2 (FGFR2) located in the neighbouring outer root sheath of keratinocytes [6]. Studies showed induction of β-Catenin and upregulation of Shh by FGF10 and consequently activation of

Wnt signalling pathway. As a result, **hair growth is promoted due to inducing and prolonging the anagen phase of hair follicles** [6,7]. Mesenchymal dermal papilla cells secrete signalling molecules, including FGF10, that can activate the Wnt pathway or, alternatively, help maintain hair follicle stem cells (HFSCs) in a quiescent state. HFSCs can proliferate into the hair germ after their activation during the anagen phase. As the cells differentiate, the hair shaft is formed and continues to elongate [7].

FGF10-STAB®

Fibroblast growth factors, including wild-type FGF10, are inherently unstable, which significantly limits their use in industry, medicine, and cosmetics. However, Enantis has developed a **stabilized form of FGF10 called FGF10-STAB®**. Through protein engineering methods, 4 amino acids of the FGF10 amino acid chain have been substituted with different residues, resulting in a **19°C increase in the protein's melting temperature** [8,9]. The higher melting temperature of **FGF10-STAB® extends the stability to more than 8 months at room temperature**, while the concentration of native FGF10 drops to 20 % in three weeks. The stabilization of FGF10-STAB® is achieved directly within the protein structure without the use of additional stabilizing agents while preserving the same biological activity. FGF10-STAB® brings economic benefits to hair care products manufacturers due to significantly slower degradation during production, storage, transportation, and after package opening. The higher stability of FGF10-STAB® also allows the preparation of final hair care formulations without stabilizing agents, reducing the risk of undesired (allergic) reactions.

Application

As a consequence of both aging and stress, the number of people suffering from hair loss has increased. This problem affects up to 50% of men and women during their lifetime [10]. Instead of undergoing procedures such as use hair transplantation surgery or drug injections (Minoxidil), often steroids, hair growth can be promoted through the application of FGF10-STAB®. The use of FGF10-STAB® in hair care involves the induction of proliferation in hair follicle stem cells, making it a promising ingredient for stimulating hair growth and treatment of hair loss [4]. Maintaining labile FGF10 in hair products active is nearly impossible, and that is why the highly stable growth factor in the form of FGF10-STAB® represents a revolutionary hair care ingredient.

FGF10-STAB® specification

INCI	AQUA SODIUM CHLORIDE DIPOTASSIUM PHOSPHATE MONOPOTASSIUM PHOSPHATE SH-POLYPEPTIDE-10
CAS NUMBER	67254-75-5
SOURCE	Bacteria *Escherichia coli* BL21 (DE3)
FERMENTATION MEDIA	Glycerol, $(NH_4)_2HPO_4$, KH_2PO_4, citric acid, $MgSO_4 \cdot 7H_2O$, Fe(III) citrate, $Na_2MoO_4 \cdot 2H_2O$, $CoCl_2 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $CuCl_2 \cdot 2H_2O$, H_3BO3, $Zn(CH_3COOH)_2 \cdot 2H_2O$
APPEARANCE	Colourless aqueous solution
BUFFER	250 mM NaCl, 16.4 mM K_2HPO_4, 3.6 mM KH_2PO_4, pH 7.5
PURITY	>95% by SDS PAGE (reduced conditions)
MOLECULAR WEIGHT	21.5 kDa
NUMBER OF AMINO ACIDS	190
ISOELECTRIC POINT	9.88
PH OF FGF10-STAB®	7.5
PH FOR FORMULATION	5.5–8.5 (for the final cosmetic formulation)
ENDOTOXINS	Endotoxin levels <0.1 ng/µg protein (<1 EU/µg) by LAL test
BIOLOGICAL ACTIVITY	ED50 ≤3.0 ng/ml, cell proliferation assay using HaCaT cells
SOLUBILITY	Water soluble

Composition of FGF10-STAB®

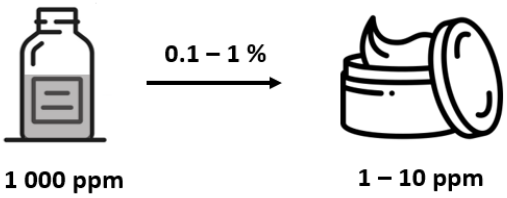
COMPONENT	INCI	ORIGIN	%
Water	AQUA	Mineral	98.10
250 mM NaCl	SODIUM CHLORIDE	Mineral	1.46
16.4 mM K_2HPO_4	DIPOTASSIUM PHOSPHATE	Mineral	0.29
1 mg/mL FGF10-STAB®	SH-POLYPEPTIDE-10	Fermentation	0.10
3.6 mM KH_2PO_4	MONOPOTASSIUM PHOSPHATE	Mineral	0.05
Total			100

FGF10-STAB® production pipeline

FGF10-STAB® is a recombinant protein produced in bacterial *E. coli* strain – BL21 (DE3) by microbial fermentation in an animal-free, non-GMO Luria Bertani media. All genetic material from the bacteria is removed upon purification process and FGF10-STAB® comes as an animal-free **liquid solution** or alternatively as a lyophilized powder[1]. Salts in the final solution have mineral origin from mines (NaCl) or mineral-derived origin (K_2HPO_4 and KH_2PO_4).

FGF10-STAB® dosing and recommended concentration

Use 0.1–1 % of FGF10-STAB® in the final cosmetic product to achieve 1–10 ppm concentration.



[1] Available for extra charge.

FGF10-STAB® formulation and recommended conditions

FGF10-STAB® can be added to the **aqueous phase** of the final cosmetic formulation, which may then be heated to a maximum of **60 °C** for up to **30 minutes**. FGF10-STAB® can be incorporated into cosmetic formulations such as **hair tonics**, **serums**, **masks**, and **eyelash serums.**

FGF10-STAB® shelf-life

Storage/Stability: Room temperature to 4 °C for at least 8 months[i].

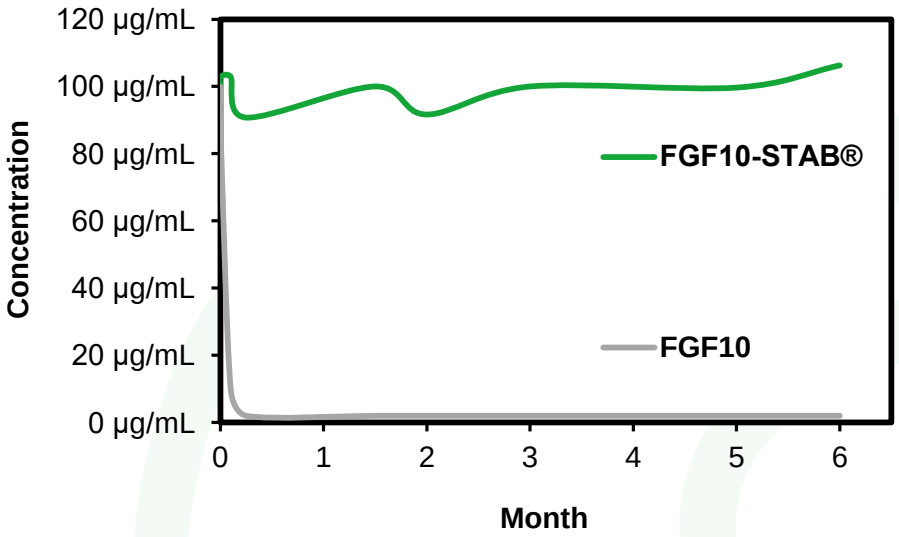
[i] *Ongoing long-term stability testing up to 2 years.*

FGF10-STAB® vs FGF10 comparison in the aqueous solution

	FGF10	FGF10-STAB®	FGF10-STAB® BENEFITS
ACTIVITY AT 37 ˚C	<2 hours	>24 hours	More stable during biological processes
HALF-LIFE AT 37 ˚C	<11 hours	>24 hours	More stable during biological processes
PROTEOLYSIS AT 37 ˚C	<10 minutes	>1 hour	More stable during biological processes
STABILITY AT 25 ˚C	<10 weeks	>8 months	Stable for a longer period (transport and storage)
STABILITY AT 40 ˚C	<1 week	6 months	Stable for a longer period (transport and storage)
ACTIVITY (ED_{50})	0.8 ng/mL	0.2 ng/mL	Lower concentration required (reduced costs)
MELTING TEMPERATURE	45 ˚C	64 ˚C	Stable at higher temperature (heating during production)

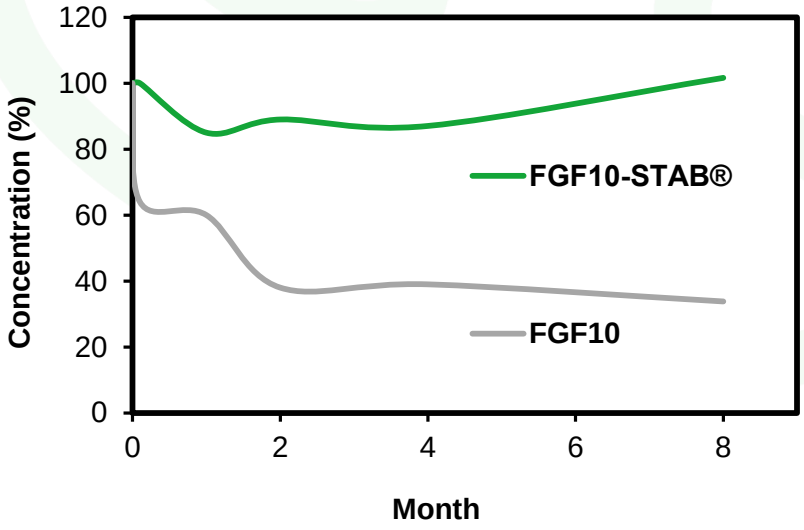
Long-term FGF10-STAB® stability in the aqueous solution at 40 °C

Concentration of native FGF10 drops to less than 10 % in 1 week at 40 °C while concentration of FGF10-STAB® is stable for at 6 months. Concentration was detected by ELISA using a specific antibody recognizing FGF10-STAB®.



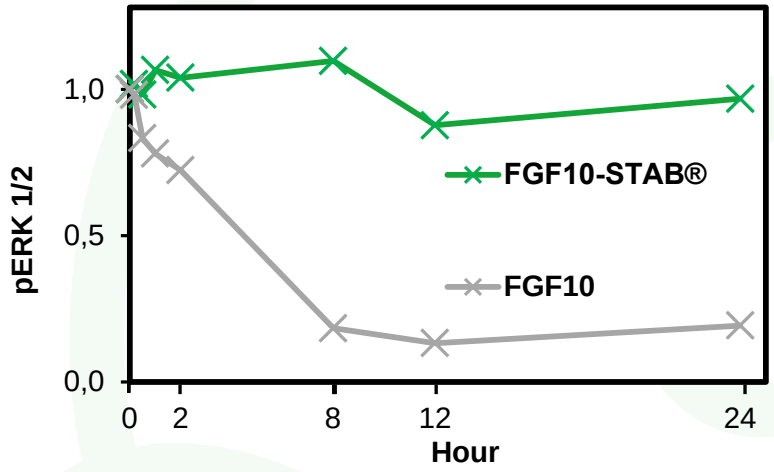
Long-term FGF10-STAB® stability in the aqueous solution at 25 °C

Concentration of native FGF10 drops to 60% in 1 week and to 40% in 8 weeks at room temperature. Concentration of FGF10-STAB® is stable for at least 8 months (ongoing long-term stability experiment). Concentration was detected by ELISA using a specific antibody recognizing FGF10-STAB®.



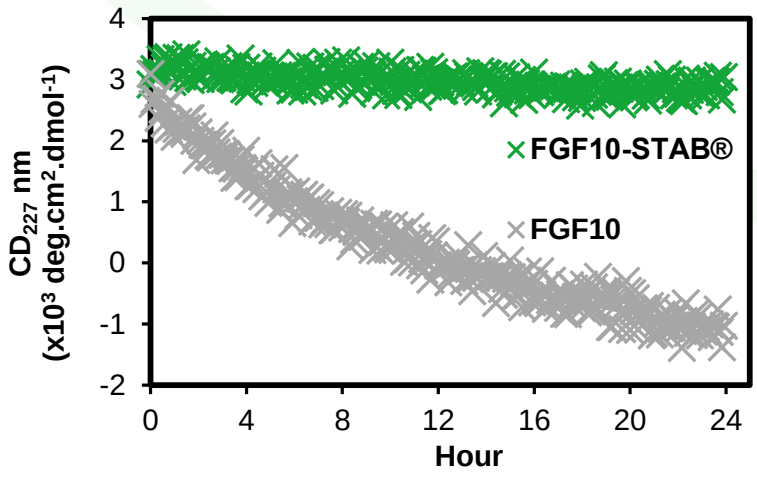
Retained biological activity of FGF10-STAB® in the aqueous solution at 37 °C

The resistance of FGF10-STAB® to higher temperatures results in **a significantly longer half-life** compared to unstable FGF10. The biological activity of native FGF10 decreases with thermal preincubation after 2 hours. The stabilized variant **FGF10-STAB® maintains complete biological activity at least 24 hours**. Experimental data were obtained through quantitative detection of the phosphorylated form of the ERK-1/2 downstream signalling pathway, which is regulated by FGF2. Quantification was performed using the western blotting from the lysate of MCF-7 epithelial cancer cell line [8,9].



Long-term FGF10-STAB® half-life in the aqueous solution at 37 °C

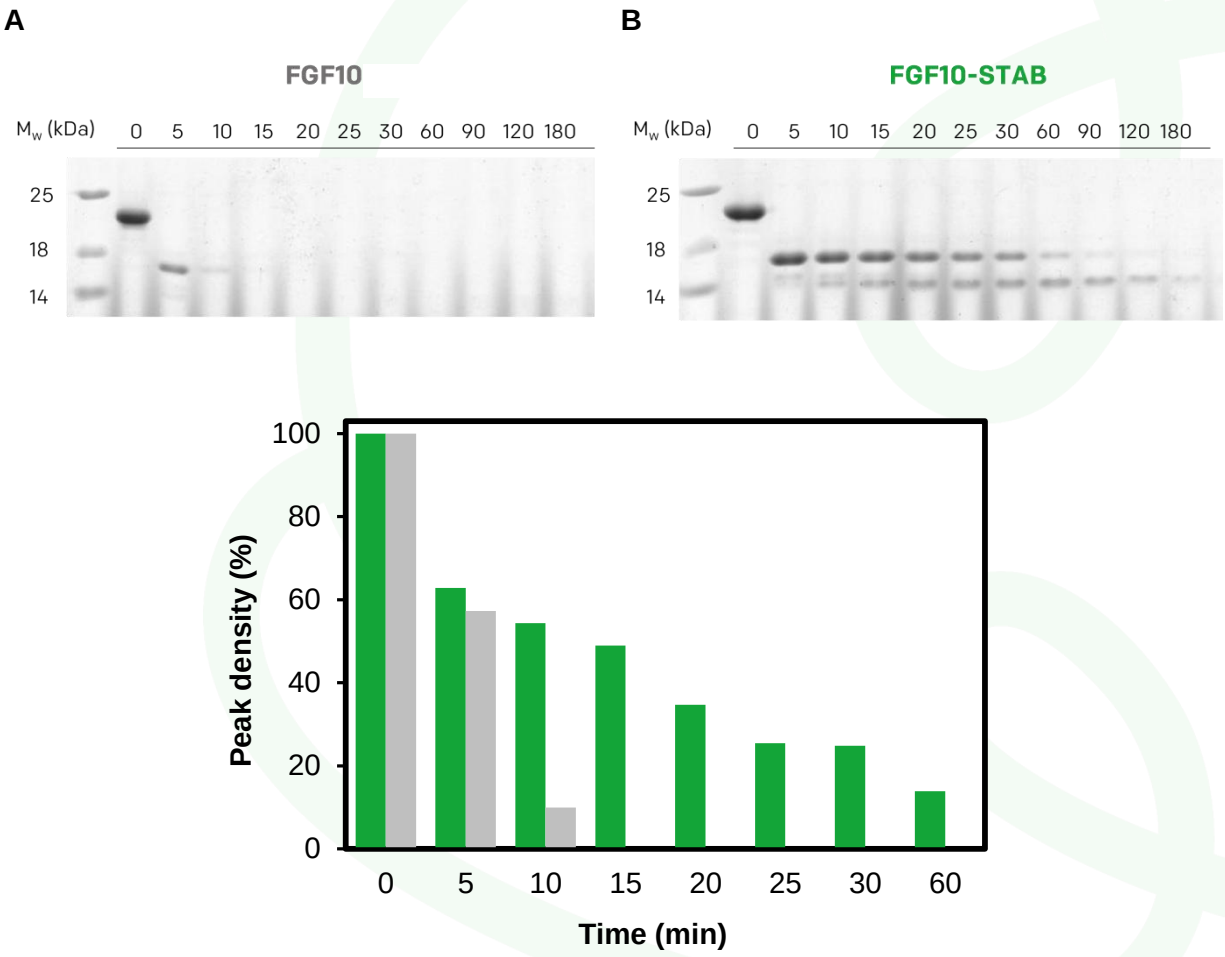
FGF10-STAB® retains its secondary structure during prolonged incubation at 37 °C. Determined **half-life of FGF10-STAB® is more than 24 hours**, while the native FGF10 is stable less than 10 hours. Changes in the secondary structure of both proteins were determined by CD spectroscopy [8,9].



Higher FGF10-STAB® resistance to proteolytic degradation at 37 °C

Endogenous skin proteases with trypsin-like activity, such as kallikrein-5 (KLK5) and kallikrein-7 (KLK7), are key enzymes involved in maintaining skin homeostasis, especially in the stratum corneum [11]. **FGF10-STAB® is more resistant to proteolytic trypsin degradation** (more than 1 hour) in comparison to native FGF10 (less than 10 minutes) [8]. The proteolytic degradation is connected with rapid loss of its biological activity [12].

FGF10 **(A)** and FGF10-STAB **(B)** in solutions were digested at 37 °C by trypsin (in a final concentration of 2 µg protease/100 µg FGF10) for 180 minutes. Products of the reaction were visualized by SDS-PAGE [8]. Peak density was quantified by ImageJ software.



Heavy metals content in FGF10-STAB®

FGF10-STAB® Cosmetics ingredient complies with the EU Cosmetics Regulation (EC No 1223/2009) and **does not exceed** the specified heavy metals limits.

The presence of heavy metals in FGF10-STAB® Cosmetics was determined by ICP/MS in the *Laboratory of atomic spectrochemistry, Masaryk University, Brno, Czech Republic*.

ELEMENT	FGF10-STAB®	LIMIT*
Lead (Pb)	0.144 ppm	2 ppm
Cadmium (Cd)	0.002 ppm	0.1 ppm
Nickel (Ni)	0.834 ppm	10 ppm
Mercury (Hg)	Not detected	0.1 ppm
Antimony (Sb)	Not detected	0.5 ppm
Chromium (Cr)	0.293 ppm	1 ppm

** Limits for final cosmetic products*

ISO 16128 Natural origin index calculation for FGF10-STAB®

Based on the ISO 16128-1:2016 (Definitions for ingredients) and ISO 16128-2:2017 (Criteria for ingredients and products), FGF10-STAB® **is composed of natural origin components** and FGF10-STAB® has **Natural Origin Index (NOI) = 1** and therefore it is compliant with ISO 16128.

COMPONENT	ORIGIN	MOLECULAR WEIGHT	WEIGHT PER 1 L
1 mg/mL FGF10-STAB®	Fermentation	21468.17 g/mol	1 g
250 mM NaCl	Mineral	58.44 g/mol	14.61 g
16.4 mM K_2HPO_4	Mineral	174.18 g/mol	2.855 g
3.6 mM KH_2PO_4	Mineral	136.09 g/mol	0.490 g
Total			**18.955 g**

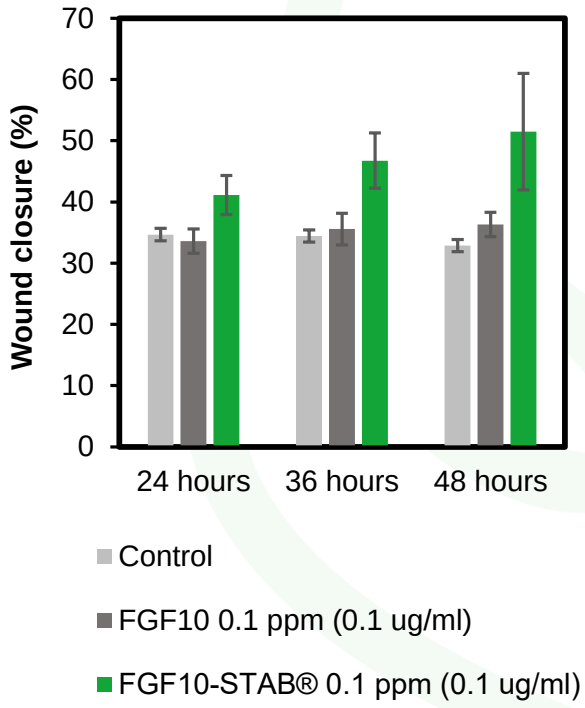
In vitro EFFICACY RESULTS

Increased cell activation and migration of human keratinocyte cells

FGF10-STAB® promotes **cell activation and migration (scratch test),** which lead to repopulation of the decellularized areas **in a shorter period of time** (higher efficiency) compared to FGF10 in the human keratinocytes scratch test.

Cells were cultured to confluence. The scratch was made using the IncuCyte WoundMaker, followed by treatment with **0.1 µg/ml (A)** and **1 µg/ml (B) of FGF10 and FGF10-STAB®**, respectively. Pictures of the migrating cells were taken and analysed and quantified by IncuCyte Cell Migration system to determine the reduction of the scratched areas and monitor the cell migration.

A



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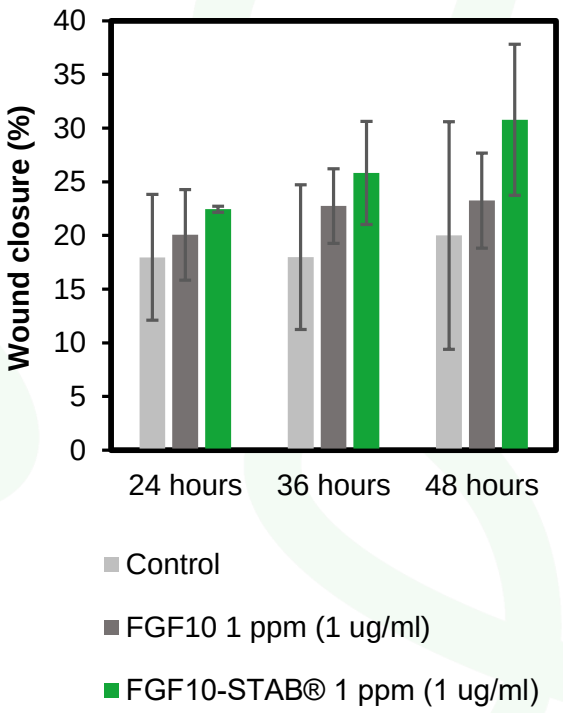
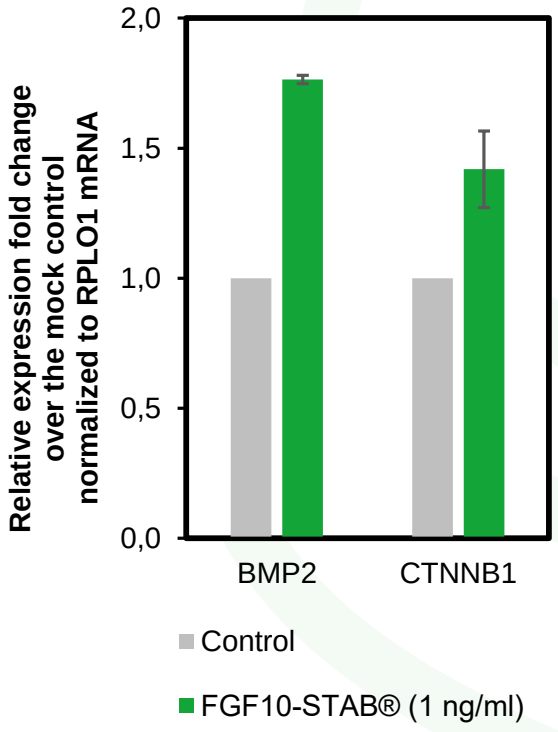


Gene expression analysis in human keratinocyte cells

FGF10-STAB® **upregulates gene expression** of **BMP2** (Bone Morphogenetic Protein 2) and **CTNNB1** (Catenin Beta 1) in human keratinocyte cells (HaCaT). BMP2 is a key signalling molecule that **regulates follicular differentiation** and **development** by modulating cellular commitment and tissue morphogenesis, supporting **healthy** and **strong follicles**. Activation of CTNNB1 **stimulates Wnt signalling**, which promotes keratinocyte proliferation, stem cell maintenance, and **hair follicle regeneration**, thereby contributing to **hair growth** and helping **restore hair density**.

Cells were cultured in starvation medium and treated after 24 hours with **1 µg/mL (A)** and **10 µg/mL (B)** of FGF10-STAB®. After another 24 hours, both treated and untreated cells (control) were harvested for gene expression analysis by RT-qPCR.

A



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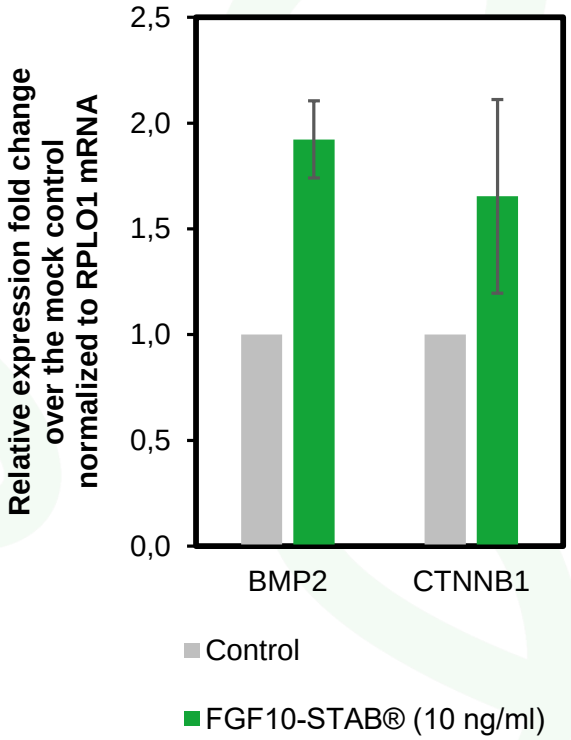

EU COMPLIANCE

EU Regulation No. 1223/2009

FGF10-STAB® **is compliant** with EU Regulation No. 1223/2009, including technical documentation, safety information and MSDS.

FGF10-STAB® (INCI: SH-POLYPEPTIDE-10) is not listed as a restricted substance in Annex III of EU Regulation No. 1223/2009.

EU Allergens

In compliance with **EU Regulation No 1169/2011**, FGF10-STAB® **does not** contain any allergens listed in Annex II.

Annex II Allergens:

- Cereals containing gluten: namely wheat (including specific varieties like spelt and Khorasan), rye, barley, oats and their hybridised strains) and products thereof.
- Crustaceans and products thereof (for example prawns, lobster, crabs and crayfish)
- Egg and products thereof
- Fish and products thereof
- Peanut and products thereof
- Soybeans and products thereof
- Milk and products thereof (including lactose)
- Nuts: namely almond, hazelnut, walnut, cashew, pecan nut, Brazil nut, pistachio nut and Macadamia nut (Queensland nut) and products thereof
- Celery and products thereof
- Mustard and products thereof
- Sesame seeds and products thereof
- Sulphur dioxide and sulphites (at concentrations of more than 10mg/kg or 10mg/L in terms of the total SO2 which are to be calculated for products as proposed ready for consumption or as reconstituted according to the instructions of the manufacturers)
- Lupin and products thereof
- Molluscs and products thereof (for example clams, oysters, scallops, snails and squid)

SAFETY DATA

In vitro cytotoxicity

The toxicological profile of FGF10-STAB® was assessed through *in vitro* testing using the MTT cytotoxicity assay (HaCaT, human keratinocyte cells). In accordance with the standards specified in ČSN EN ISO 10993-5, it was demonstrated that **FGF10-STAB® at a concentration of 250 µg/ml did not exhibit cytotoxic potential under the chosen test conditions.** The data are available upon request.

In vitro skin irritation

FGF10-STAB® irritation potential was tested in compliance with OECD TG 439 – In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method. Skin irritation by the test sample was assessed using the MTT cytotoxicity assay on the EpiDerm skin model, an organotypic model of human epidermis consisting of reconstructed epidermis and functional stratum corneum. The results demonstrated that **FGF10-STAB® at a concentration of 1000 µg/ml may be classified as "no category of skin irritation" under the UN Globally Harmonized System of Classification and Labeling of Chemicals**, based on the selected test conditions. The complete test protocol is available upon request.

SAFETY EVALUATION IN HAIR PRODUCT

In vivo tests

No observed adverse effect of (NOAEL) – for KGF 2 (Repifermin) = 50 µg/kg/day (human, intravenously, 28 days) [13].

Margin of Safety (MoS) assessment

Expected skin exposure area = 1 010 cm^2, body weight of the individual = 60 kg [14]

Daily exposure dose = 1 000 mg (applied once per day)

Relative daily exposition ($E_{product}$)= 16.7 mg/kg body weight/day

$$\left(E_{product} = \frac{daily\ exposition\ dose}{human\ body\ weight} = \frac{1000}{60}\right)$$

Dermal absorption (DA_p) = 0.6% (data for transdermal penetration of similar molecule - FGF2-STAB through the STRAT-M membrane)

Substances > 500 Da = very low dermal absorption => $DA_p \approx 50\%$ [14]

Recommended concentration of FGF10-STAB® in the hair product = 10 µg/ml (final concentration)

Retention factor according to the product type (hair styling product) = 0.1 [14]

The systemic exposure dose (SED) considering the 0.6% dermal absorption is calculated as 0.0000001 mg/kg body weight/day.

$$\left(SED = E_{product}\ x\ \frac{C}{100} x\ \frac{DA_p}{100} x\ f_{ret} = 16.7\ x \frac{0.001}{100} x\ \frac{0.6}{100} x\ 0.1\right)$$

The systemic exposure dose (SED) considering the 50% dermal absorption is calculated as 0.00000835 mg/kg body weight/day.

The Margin of Safety (MoS) when considering a 0.6% dermal absorption and utilizing the dose with no apparent toxic effect, extra default value (50%) due to no oral bioavailability data and default factor 3 (no 90-day study, but only 28-day) in the calculation is 85 000.

$$\left(MoS = \frac{NOAEL}{SED} x\ \frac{1}{2} x\ \frac{1}{3} = \frac{50\ x\ 10^{-3}}{0.0000001} x\ \frac{1}{2} x\ \frac{1}{3}\right)$$

The Margin of Safety (MoS) when considering a 50% dermal absorption and utilizing the dose with no apparent toxic effect, extra default value (50%) due to no oral bioavailability data and default factor 3 (no 90-day study, but only 28-day) in the calculation is 998 => cosmetic ingredient is considered as safe, if MoS ≥ 10 (reference MoS = assessment factors for extrapolation, and consideration of human variability).

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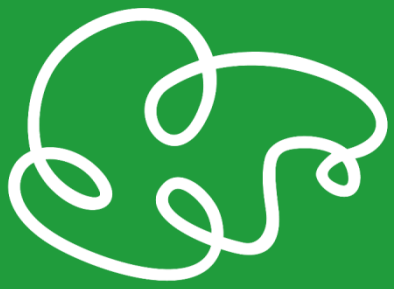

Enantis

Enantis is an innovative biotechnology company focused primarily on **growth factors**, which are among the most important biomolecules in the human body. Our goal is to **enhance their low stability**, making them more applicable in industries such as **cosmetics and medicine**.

The core values and uniqueness of Enantis lie in the **full integration of the development process**, from designing novel molecules to their optimization, characterization, and production. Our mission is to contribute to a **healthier and more sustainable future** through the development of innovative solutions.

Enantis s.r.o.

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