

Active Beauty
Brightenyl®
Bio-boosted skin brightening
and color correction



Crafted by white and green technologies



Focus on the product

Skin color and pigmentation: a multilayered process

The color of our skin depends on several biological factors:

- ▶ The activation of keratinocytes by UV rays and inflammation trigger the production of melanin by melanocytes, which will migrate to the upper layers of the skin.
- ▶ The localised inflammation influences the redness of the skin.

An efficient control of skin color must address this multilayered process in order to provide safe and sustainable results.

Stratum microbium: an active living veil on our skin

Our skin lives in symbiosis with massive amounts of microorganisms called the human skin microbiote. This innate and beneficial microbial population represents 100 to 10,000,000 microorganisms by skin cm² depending on area¹. The skin microbiote is continuously communicating with our epidermal cells, generating metabolites and stimulating their cellular interactions.

Metagenomic, a DNA technology which enables the analysis of microbial genomes without cultivating microorganisms, has recently proven that skin microbiote expresses alpha-glucosidases: enzymes that activate specific cosmetic compounds into biologically active molecules on the skin surface.

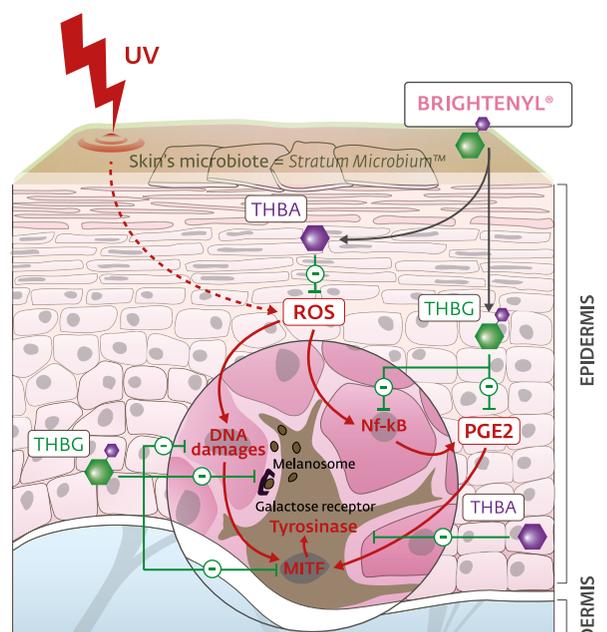
Brightenyl®: skin microbiote activated whitening

Brightenyl® is a new generation of skin melanoregulator molecule which is activated by the *Stratum Microbium*®. It contains the patented THBG ingredient (Tri Hydroxy Benzoic acid alpha-Glucosides).

Once applied on skin, THBG is partially converted by the skin microbiote into THBA (Tri Hydroxy Benzoic Acid - a natural inhibitor of tyrosinase), which acts in synergy with THBG to control the skin color.

THBG and THBA cooperate at 7 levels to whiten and uniformise the skin color:

1. It inhibits ROS production
2. It prevents UV-induced DNA damages
3. It controls the expression of MITF
 - ▶ STOPS the melanogenesis process
4. It reduces the expression of PGE2
 - ▶ DECREASES vasodilation and redness
5. It controls the Nf-kB pathway
 - ▶ REDUCES inflammation
6. It saturates keratinocytes receptors
 - ▶ STOPS melanin transfer
7. It blocks melanin synthesis even under UV conditions
 - ▶ WHITENS the skin



¹ Mathieu et al, Research in Microbiology 2013

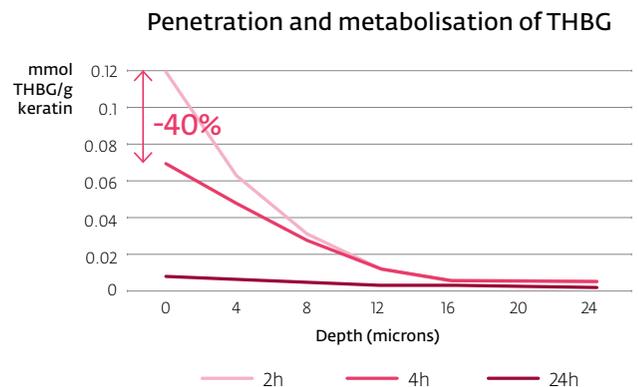
Biological activity

Bio-activation by the *Stratum Microbium*[®]

1. Skin penetration and transformation of THBG (Clinical - Raman spectroscopy)

Raman spectroscopy was used on 3 volunteers to evaluate the penetration of THBG. THBG at 5% in water has been applied on forearms on an area of 4 cm². The penetration depth of the molecule into the *stratum corneum* was measured 2h, 4h, and 24h after application.

Results: THBG penetrates quickly into the upper layers of the skin up to 12 µm of depth, corresponding to the *stratum corneum* containing the skin microbiote. It disappears quickly with 40% of the molecule being converted between 2 and 4 hours after application.



2. DNA sequencing and analysis of skin microbiote (metagenomic & bioinformatic test)

24 samples of skin's microbiote were done on 2 volunteers with a cotton impregnated with NaCl. The total microbial metagenomic DNA was extracted, and analysed by high throughput sequencing (illumina HiSeq[®]). Sequences were processed to discover which genes can be expressed by the human skin microbiote.

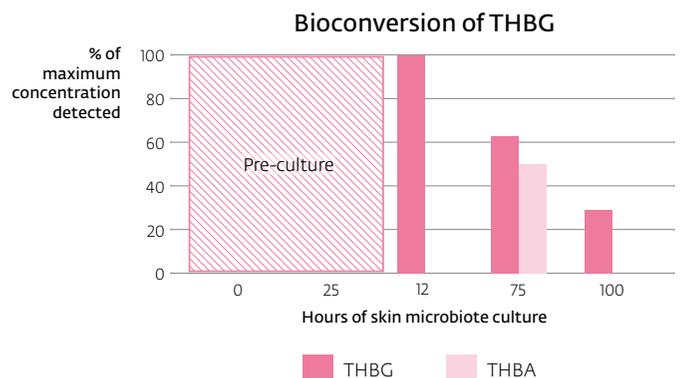
Results: 40 billions of DNA bases were analysed corresponding to 15,600 microbial genomes (*S. epidermidis* equivalent). Revealing 122 alpha-glucosidases genes from 20 microbial species, suggesting the role of the human skin microflora in the conversion of THBG into THBA.



3. Bio-activation of THBG into THBA by the *Stratum Microbium* (in vitro test)

A sampling of skin's microbiote was done on 9 volunteers with a swab impregnated with NaCl on 3 areas: forehead, cheek and forearm. Microbiote was cultured in liquid medium (HT medium) at 30°C in presence of THBG. Supernatants were collected upon time and analysed by HPLC to detect THBG and THBA.

Results: The *Stratum Microbium*[®] partially converts THBG into THBA by removing the alpha-glucoside moiety of THBG.



Biological activity

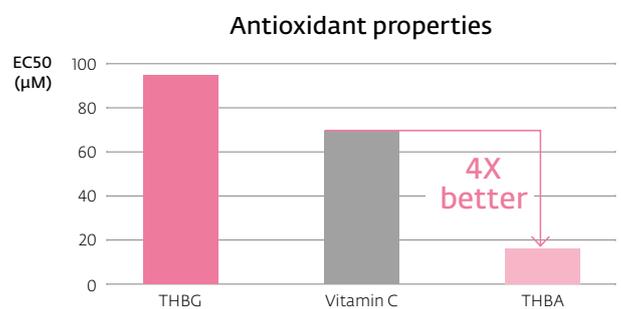
Protective effect of Brightenyl® (in vitro)

1. Antioxidant effect of Brightenyl®

THBG and its microbiote activated derivative THBA were evaluated for their antioxidant property using the DPPH assay. They were compared with vitamin C. The anti-radical activity of the compounds was expressed as a decrease of concentration of DPPH or as EC50 (concentration of a compound decreasing the absorbance of a DPPH solution by 50%).

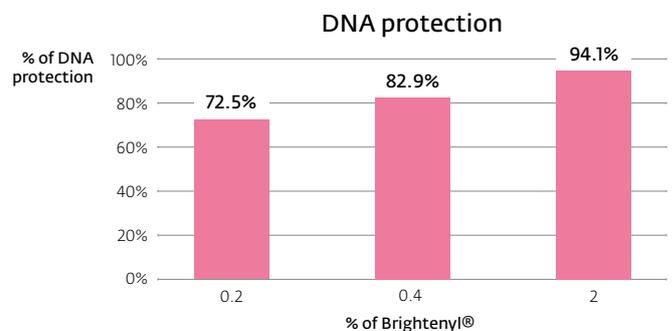
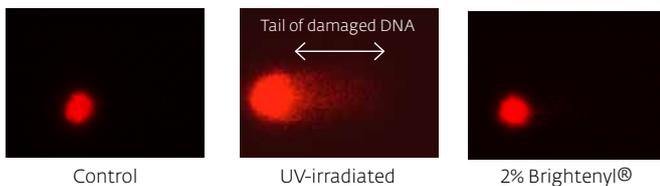
Results: Brightenyl® has an instant antioxidant activity.

This free radical scavenging activity is amplified by skin microbiote upon conversion of THBG into THBA showing a 4x better antioxidant efficacy compared to Vitamin C.



2. DNA protection by Brightenyl®

Brightenyl® was tested for its photo-protection activity using the Comet assay on melanocytes. This test measures the degradation of DNA in skin cells exposed to solar irradiation. Brightenyl® has been tested at three concentrations: 0.2%, 0.4% and 2%.

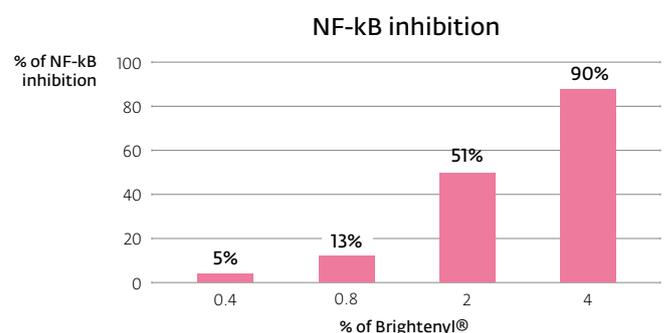


Results: Brightenyl® shows an excellent DNA protection with up to **94% of photo-protection** at 2%.

3. Control of skin inflammation

Brightenyl® was tested for its anti-inflammatory property. Human cells were stressed with IL-1 β inducing a pro-inflammatory NF-kB response. THBG was tested at 0.4%, 0.8%, 2% and 4% to evaluate its inhibition effect on the NF-kB pathway.

Results: Brightenyl® inhibits **NF-kB response up to 90%** at 4%.



Biological activity

Brightenyl®: multilevel melanogenesis inhibition (*ex vivo* & *in vitro*)

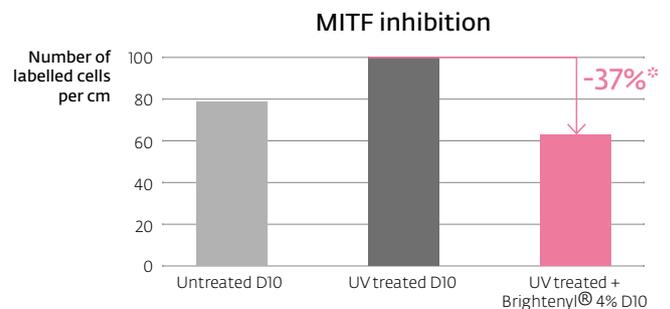
Studies were run to confirm Brightenyl®'s mode of action. 3 markers were evaluated: MITF expression, PGE2 expression and inhibition of galactose receptors; all linked with the production of melanin. *Ex vivo* studies were done on an abdominal-plasty from a 43 years old caucasian woman (skin phototype III to IV). Brightenyl® was tested at 4%. Products were applied on explants 30 min before irradiation. They were irradiated at the dose of 1.125J/cm² UVA, (6-8% of UVB) daily during 10 days (equivalent to daily exposure). Histological and immuno-histological analysis were run.

1. Decrease of MITF expression

MITF, the transcription factor involved in the genesis of melanin synthesis pathway, was observed with an antibody and then quantified with Cell^AD device. MITF positive cells were counted per cm of epidermis.

Results: Brightenyl® at 4% **reduces MITF expression** under UV conditions by **-37%***.

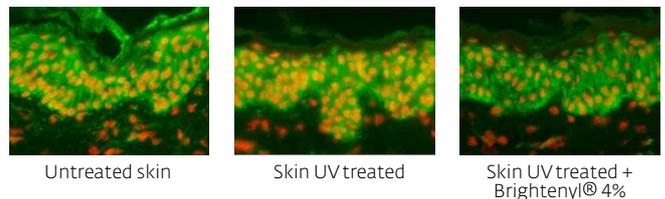
* p<0.01 compared to UV-treated, Student's t-test



2. Decrease of PGE2 production

Prostaglandin-E2 is produced by adult human epidermal keratinocytes in response to UVB. PGE2 stimulates the formation of dendrites in melanocytes². PGE2 expression under UV conditions was observed with an antibody (detection of green fluorescence).

Results: Brightenyl® at 4% visibly **reduces PGE2 expression** under UV conditions.



3. Reduction of melanin content

Melanin was visualised with a Fontana Masson's staining and quantified after exposure to UV, to evaluate its level of production in the skin explants.

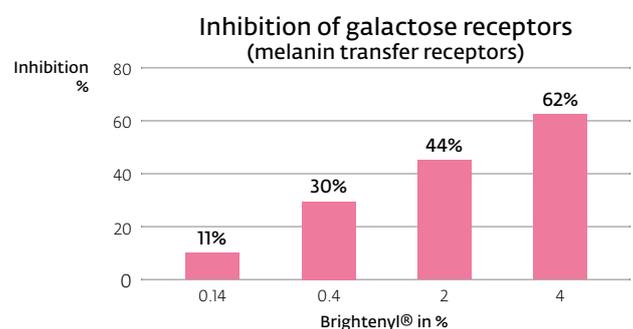
Results: Brightenyl® at 4% **reduces melanin content** in the basal layer by **-16%***.



4. Inhibition of melanin transfer receptors (*in vitro*)

The transfer of melanin from melanosomes to keratinocytes requires free galactose receptors on the surface of keratinocytes. Brightenyl® was studied for its capability to block keratinocytes galactose receptors. Increasing concentrations of Brightenyl® were tested in competition with alpha galactose-BSA on keratinocytes galactose receptors. Quantification was done by fluorescence.

Results: Brightenyl® **saturates the keratinocytes galactose receptors, up to 62%**, thereby preventing melanin transfer.



Efficacy

Brightenyl®: visible uniformity and brighter skin tone (Clinical evaluation)

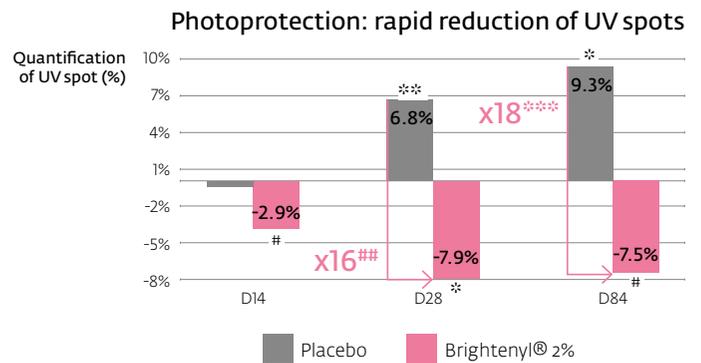
A double blind study versus a placebo under dermatological control was done on 20 Korean women aged between 30 to 60 years old with dark spots on the face. The study started at the end of summer, period during which the UV exposure is still important. They applied twice a day a placebo cream on one side of their face, and the same cream containing 2% of Brightenyl® on the other side of the face for 84 days. Skin color, brightness and redness were assessed using different equipments (Visia CR, Siascope and Chromameter).

1. UV spots reduction (Visia CR) // Uniformisation of skin tone

UV spots happen when melanin coagulates below the skin's surface because of sun exposure. They are a marker of photo damages. Reducing UV spots content enables to decrease the level of future hyperpigmented spots at the skin's surface.

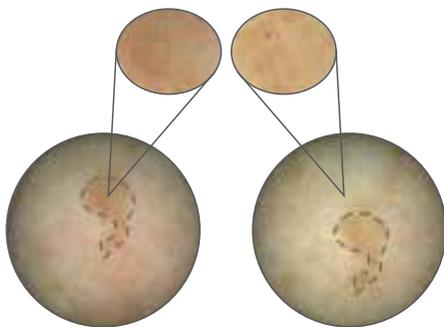
Results: Brightenyl® **decreases UV spots** in only one month compared to placebo, with up to **18 times better results after 84 days.**

- # p<0.1 compared to D0, Student's t-test
- * p<0.05 compared to D0, Student's t-test
- ** p<0.01 compared to D0, Student's t-test
- *** p<0.005 compared to placebo, Student's t-test
- ## p<0.05 compared to placebo, Student's t-test



2. Improvement of skin lightening (Siascope)

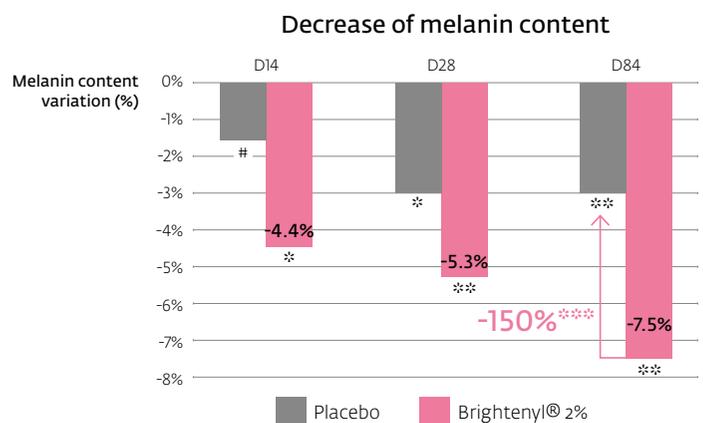
Melanin content was measured with the Siascope on a spot area. The reduction of the melanin content was observed on the skin and on the pigmented spots.



Results: Brightenyl® **decreases global melanin content** by **-150%** compared to placebo, **after 84 days.**

Skin and hyperpigmented spots are visibly whiter.

- # p<0.1 compared to D0, Student's t-test
- ** p<0.01 compared to D0, Student's t-test
- *** p<0.005 compared to placebo, Student's t-test
- * p<0.05 compared to D0, Student's t-test
- ** p<0.05 compared to placebo, Student's t-test



Efficacy

3. Decrease of skin redness (Chromameter)

The a* value corresponds to the red component of the skin. This value has been measured upon time and compared to the placebo data.

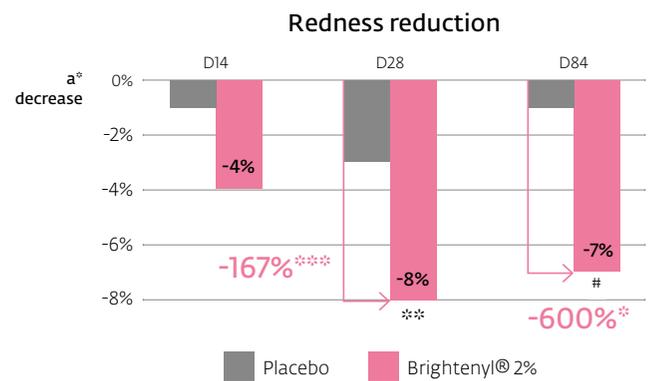
Results: Brightenyl® decreases the skin a* value by **-600%** compared to placebo, after 84 days.

p<0.1 compared to D0, Student's t-test

** p<0.01 compared to D0, Student's t-test

* p<0.0001 compared to placebo, Student's t-test

*** p<0.01 compared to placebo, Student's t-test

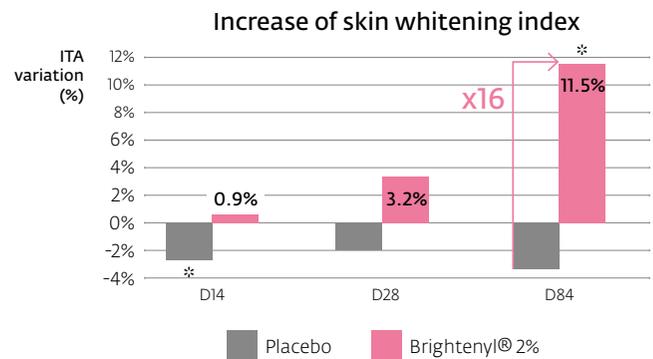


4. Increase of skin brightening (Chromameter)

ITA angle is an essential parameter related with the global brightening efficacy of a product, which takes into account the L* and b* values. The more ITA is increased, the whiter the skin is.

Results: Brightenyl® increases skin whitening index ITA by **16 times** compared to placebo, after 84 days.

* p<0.05 compared to D0, Student's t-test



Digital pictures from Visia CR

Summary



Technical information

| | |
|---------------|---|
| INCI: | Water (and) Glycerin (and) Diglucosyl Gallic Acid |
| Origin: | Biotechnology |
| Preservation: | Preservative free |
| Appearance: | Clear, yellow-amber liquid |
| Solubility: | Water soluble |
| Dosage: | 1-4% |
| Processing: | Can be added at the end of the formulation process under stirring or homogenising. Can be heated for a short time with the water phase of formulation. Formulate at temperature below 50°C, and final pH below 6.0. |

Claims

| | |
|---------------|---|
| Claims: | Skin whitening, skin color correction, redness reduction, skin brightening, skin lightening, skin tone uniformisation, reduction of pigmented spots, correction of melasma, correction of pigmentation disorders. |
| Applications: | Whitening creams, brightening serums, lightening lotions, CC creams (color correction creams), skin tone enhancing gels, anti-pigmented spots serums. |

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