

Active Beauty
Chronoglow™
Biomimetic control of epigenetics
for skin rejuvenation

Crafted by green fractionation technology



Focus on the product

Resurrection plants, a natural inspiration for skin rejuvenation

Nature has different strategies when it comes to survival of vegetal species. **Xerophyte species** can resist to long periods of drought with different mechanisms. Among those adaptation skills: a high capacity of water storage, an extensive root system, water loss prevention, or the possibility to enter a dormant phase, with a reduced photosynthesis. This is precisely the mechanism at stake in the so-called **resurrection plants**, from which we sourced our inspiration for Chronoglow™ development.



Haberlea rhodopensis is one of the few European resurrection plants, endemic from the Rhodopes mountain (Bulgaria and Greece). This *Nunatak* plant has survived the last ice age (until 10,000 thousand years ago) thanks to its ability to grow on ice-free peaks and to survive to extreme droughts, but is extremely difficult to grow and propagate, as it requires very specific conditions to blossom.

Thanks to their expertise, and with recent state of the art adaptations due to global warming, **Active Beauty botanists have been able to re-create a perfect growth environment** using indoor farming and LED technology, optimising energy by more than 80% compared to a classical greenhouse cultivation.

Studying and understanding survival capacity of *H. rhodopensis* under severe conditions was a key to imagine a unique mode of action for skin rejuvenation, bringing biomimetic cellular ageing reversal mechanisms to the beauty industry.

Epigenetic control, from plant survival to skin ageing reversal

During the metabolic screening of *H. rhodopensis*, a high concentration in a unique compound was identified: myconoside, a polyphenol glucoside, with a **strong antioxidant activity**, making this extract an exciting candidate for well-ageing applications. Going further *H. rhodopensis* is described in the literature¹ as **regulating its transcriptome during desiccation and rehydration phases**, therefore modulating its genes expression, underlying its observed physiological and metabolic responses, and their so-called "resurrection" behaviour.

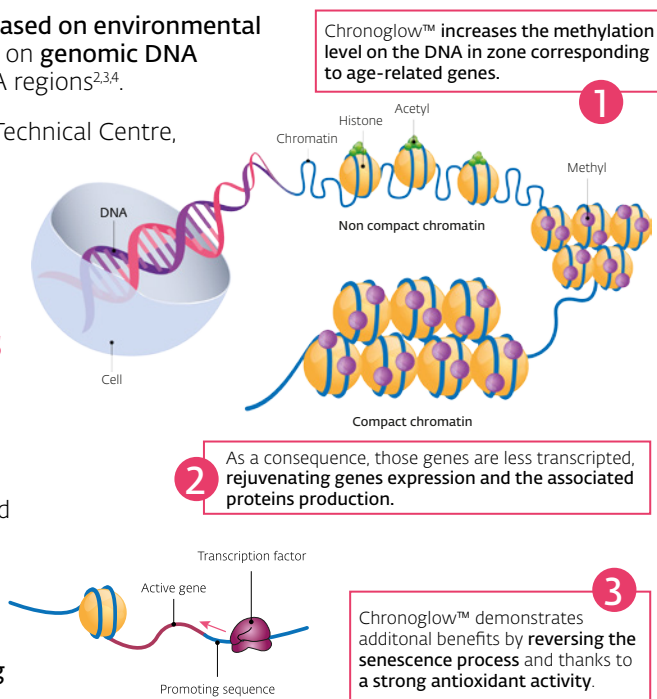
Epigenetic mechanisms are factors driving genes expression, based on environmental factors. Most of the epigenetic regulation mechanisms are based on **genomic DNA compaction**, through **acetylation** or **methylation** of specific DNA regions^{2,3,4}.

During its biological investigation scientists in our Skin Expertise Technical Centre, ***H. rhodopensis* leaf extract has demonstrated its capacity to rejuvenate the methylome (DNA methylation level).** This resulted in reactivating key genes expression and proteins production, responsible for DNA repair, antioxidant defences, or extra-cellular matrix reorganisation.

Chronoglow™, inspired by Nature's best defences mechanisms

Chronoglow™ **rejuvenates the skin at different cellular levels** (from epigenetics to proteins expression) and **protects skin cells from senescence and oxidation.** Clinical tests have demonstrated the unique benefits of Chronoglow™ obtained **in just 2 weeks, improving the skin elasticity, luminosity and radiance, with growing benefits all along the clinical test duration.**

Chronoglow™ is therefore a **powerful botanical ingredient, with exclusive benefits of well-ageing and radiance, mimicking rejuvenation mechanisms identified in resurrection plants.**



1. Liu, J. et al. Transcriptome reprogramming during severe dehydration contributes to physiological and metabolic changes in the resurrection plant *H. rhodopensis*. BMC Plant Biol 18, 351 (2018)
2. El-Osta et al. DNA Methylation and Histone Deacetylation in the Control of Gene Expression: Basic Biochemistry to Human Development and Disease. Gene expression (2000)
3. Johnson et al. The Role of DNA Methylation in Aging, Rejuvenation, and Age-Related Disease. Rejuvenation Research (2012)
4. Li et al. Chromatin higher-order structures and gene regulation. Curr Opin Genet Dev (2011)

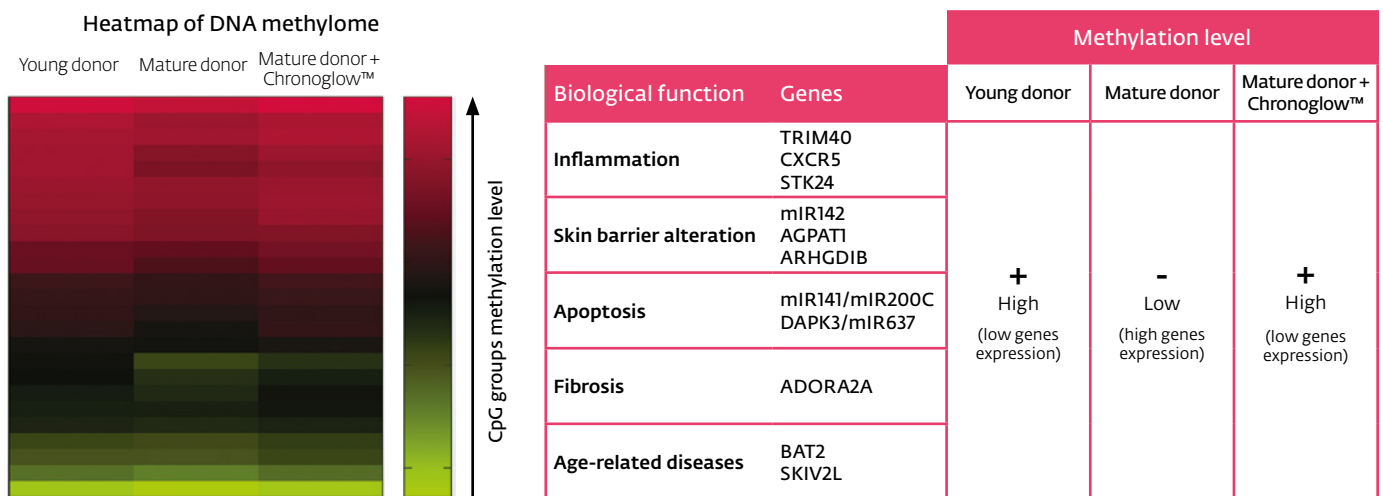
Biological activity

Multi-level rejuvenation, from methylome to proteome through transcriptome

1. Epigenetic control of skin youth through DNA methylation (*in vitro*)

Normal human dermal fibroblasts (NHDFs) from a mature donor (54 years old) were treated with Chronoglow™ at 0.2% for 36 hours or left untreated, and were compared with NHDFs from a young donor (25 years old). Genomic DNA (gDNA) was extracted from all samples and DNA methylation quantification was performed on 1386 differentially methylated regions (CpG groups).

A significant modification of methylation for untreated mature donor versus untreated young donor was observed, giving a selection of 349 CpG groups significantly impacted by skin ageing. Those 349 CpG groups were then investigated on the mature skin samples to compare their methylation level before and after the treatment with Chronoglow™.



Results: Among the CpG groups impacted by ageing, Chronoglow™ induces a significant modification of methylation level for 25 CpG groups in the mature donor ($p < 0.05$, Student's t-test), reversing it to the young donor conditions. Those groups are identified as promoters mostly linked to ageing. All of them are hyper-methylated in the young skin cells (inhibition of genes expression) and hypo-methylated in the mature skin cells (promotion of genes expression).

A deeper analysis enables to identify five biological functions driven by those CpG groups and their associated genes, all of them being over-expressed in a mature skin compared to a young skin, but significantly inhibited with the use of Chronoglow™, demonstrating a clear methylome rejuvenation.

2. Impact on genes expression (*in vitro*)

Fibroblasts from the previous study's mature donor were used to perform a transcriptomic analysis (RT-qPCR) and assess the modulation of genes expression after 48 hours of treatment with Chronoglow™ at 0.2%.

Results: Chronoglow™ stimulates genes expression involved in cellular defences, DNA repair and cell proliferation. It also modulates the extracellular matrix (ECM) components and organisation as observed by the regulation of genes involved in ECM remodelling and fibrosis, ultimately contributing to a general rejuvenation of the skin.

Biological function	Genes	Fold change
Cellular defences	SOD2	1.24**
	HSP90AA1	1.30*
DNA repair	GADD45A	1.29*
Cellular proliferation	FGF7	1.31*
Extracellular matrix	MMP3	-1.17*
	ELN	1.19*
Fibrosis	ACTA2	-1.13*

Student's t-test: #p < 0.1, *p < 0.05, **p < 0.01

Biological activity

3. Proteome rejuvenation (*ex vivo*)

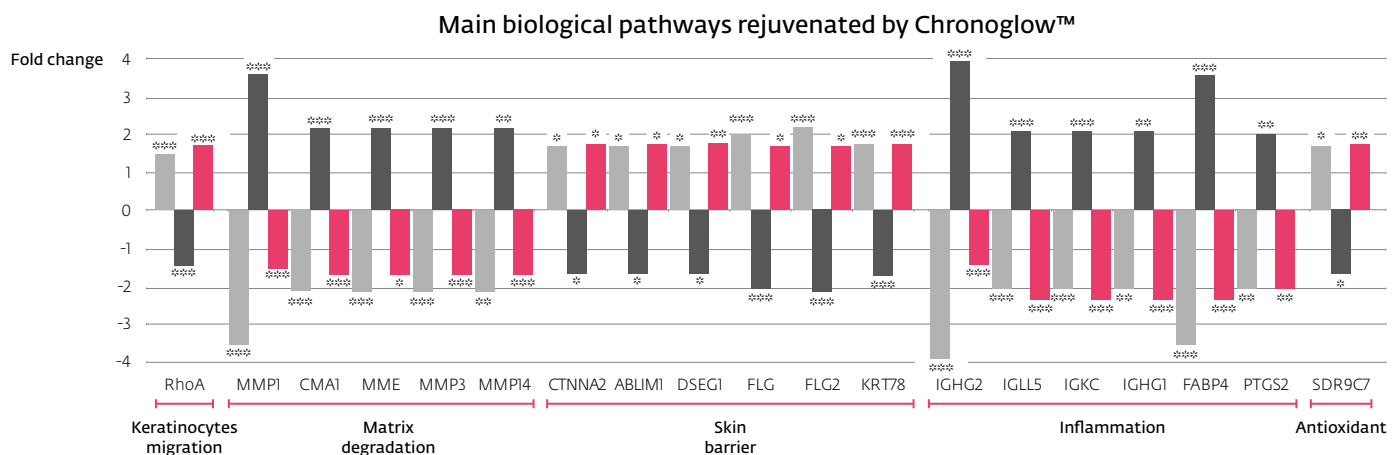
A full proteome characterisation was performed on skin explants from a mature donor (53 years) which were treated with Chronoglow™ at 3% for 5 days or left untreated and on skin explants from a young donor (18 years) which were left untreated.

All the explants were analysed to identify and quantify their full protein composition, and assess which proteins are significantly modulated by skin ageing (untreated mature donor *versus* untreated young donor) and which ones are significantly restored by the use of Chronoglow™ (treated mature donor *versus* untreated mature donor).

Results: 469 proteins (over 2,788 analysed) were found to be significantly impacted at least in one of the conditions *versus* mature skin.

Chronoglow™ significantly restores 61 (29%) of the 210 proteins impacted by ageing to a condition closer to a young skin status.

A deeper analysis enables to identify the main biological pathways reactivated by the use of Chronoglow™, two main benefits can be highlighted: skin elasticity by improving keratinocytes migration and preventing matrix degradation and **skin complexion** by improving the skin barrier function, decreasing the inflammation process and boosting antioxidant defences.



ANOVA followed by Tukey HSD test (post hoc): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Legend: Young donor (grey), Mature donor (dark grey), Mature donor + Chronoglow™ 3% (red)

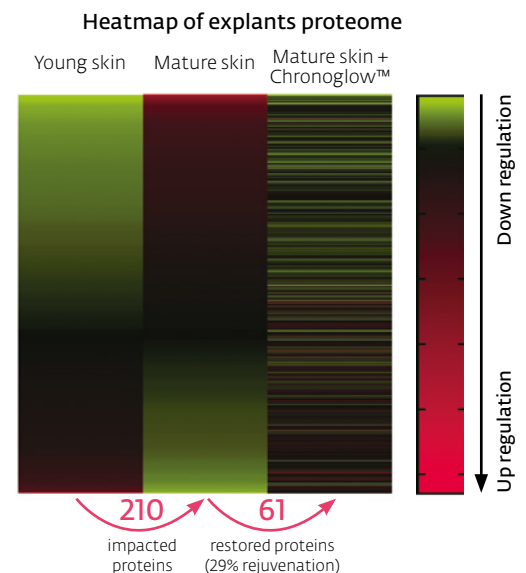
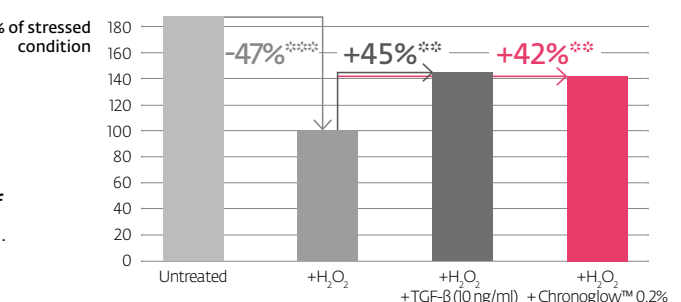
4. Boosting collagen I neosynthesis (*in vitro*)

Normal Human Dermal Fibroblasts (NHDFs) from a 28 years old donor were stressed with H_2O_2 (600 μ M for 2 hours) to mimic an oxidative stress. Cells were then treated with Chronoglow™ at 0.2% or with TGF- β as a reference. Type I pro-collagen quantification was then performed thanks to an ELISA assay.

Results: Chronoglow™ significantly protects the neosynthesis of collagen I in cells submitted to an oxidative stress, by up to +42%.

Student's t-test: ** $p < 0.01$, *** $p < 0.001$

Type I pro-collagen protection



Biological activity

Reversal of cellular senescence (*in vitro*)

A replicative senescence model was developed using human fibroblasts from a 29 years old donor. Cell senescence was induced by successive replication in culture up to passage 9. Senescent fibroblasts were then stimulated or not with Chronoglow™ at 0.2% for 48h and SA-β galactosidase activity was then measured.

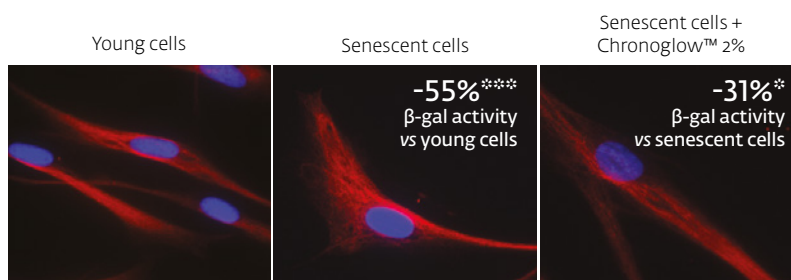
The morphological aspect of the cells was also analysed by microscopic observation after vimentin immunostaining, as it is demonstrated that senescence has a significant impact on fibroblasts morphology (enlarging them, and making them lose their fusiform aspect).

Results: Chronoglow™ significantly reduces SA-β galactosidase activity, down to -31%, efficiently reversing the cellular senescence process.

This is confirmed by the morphological observation, as Chronoglow™ enables to recover a fibroblast aspect closer to young fibroblasts after treatment of the senescent cells.

Student's t-test: *p<0.05, ***p<0.001

SA-β Galactosidase activity and fibroblasts' morphology



Antioxidant properties

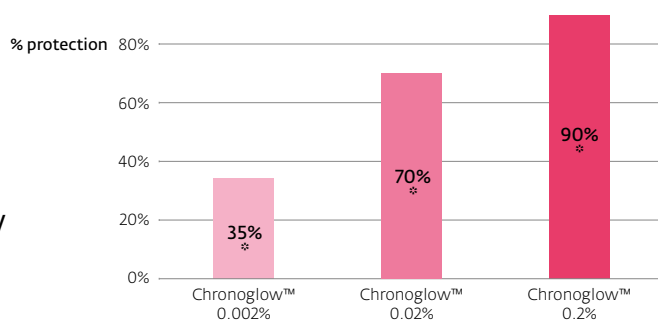
1. ROS scavenging benefits (*in vitro*)

Antioxidant evaluation was performed thanks to an AmplexRed assay kit to evaluate ROS scavenging properties of Chronoglow™ at increasing concentrations. Hydrogen peroxide was used as a source of oxidative stress to produce ROS, which were then quantified thanks to fluorescence.

Results: Chronoglow™ demonstrates a dose-dependent efficacy as an antioxidant, with a protection level up to +90% at 0.2%.

Student's t-test: *p<0.05

Oxidative protection (ROS scavenging)

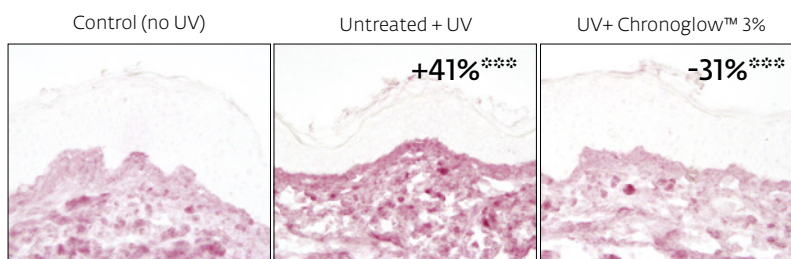


2. Reduction of oxidised proteins content (*ex vivo*)

Skin explants from a 40 years old donor were treated with 3% of Chronoglow™ or left untreated for 6 days. On day 5, the skin explants were then exposed to UVA radiation (1.5J/cm²) and UVB radiations (20 mJ/cm²). The protein oxidation level was then measured using Immunostaining on the 6th day.

Results: Chronoglow™ at 3% induces a significant reduction of oxidised proteins production under UV radiations, down to basal level (non-irradiated).

Unpaired student's t-test ***p<0.001



Efficacy

Improvement of skin firmness and radiance (clinical evaluation)

To evaluate the benefits of Chronoglow™ in face care applications, a clinical test was conducted in double blind versus placebo. This study was carried out on 20 women (from 35 to 65 years old), selected for their dull skin tone. Panellists applied a cream containing 3% of Chronoglow™ or a placebo (same formula without the active) on hemiface, once a day, for 60 days.

Skin elasticity and skin tone were measured at D0, and after 15, 30 and 60 days of treatment to evaluate the benefits of Chronoglow™ and compare it to the placebo.

Thanks to its potent biological activity detected in the previous biological studies, Chronoglow™ is able to significantly improve skin elasticity in just 2 weeks, with a prolonged efficacy during the 2 months trial period. It ultimately contributes to a global improvement of the skin tone (through luminosity and radiance/gloss), with an increasing efficacy during the whole clinical test period.

To better represent the results and get a better perception of the efficacy, an artificial intelligence enabled the generation of a neutral avatar obtained by mixing several faces giving an "average" face for both products at the various times of measurement, obtaining a visual representation of the measured parameters.

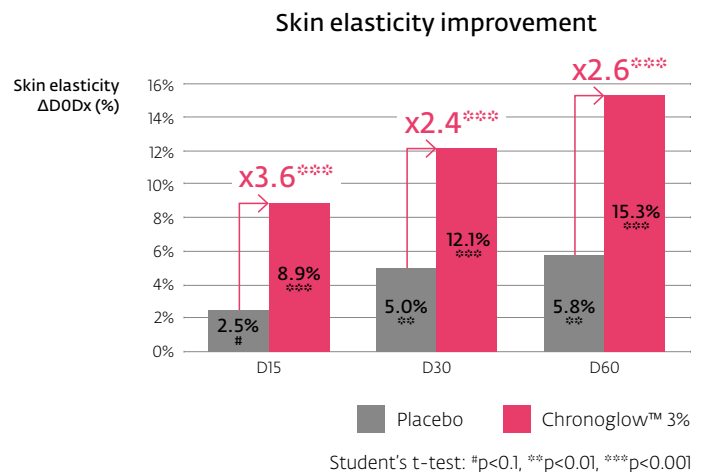
1. Skin elasticity enhancement

Skin elasticity was determined using the suction/elongation method thanks to a Cutometer®.

The parameter which was followed was the overall elasticity (R2), which represents the ability of the skin to return to its original rest position after a stressful event. The higher is the value, the better is the skin elasticity.

Results: Chronoglow™ significantly improves skin elasticity, already after 2 weeks of use (by up to +8.9%, more than 3 times better than the placebo).

This positive impact on the skin elasticity is increasing during the whole duration of the clinical test, with an increase up to +15.3% after 2 months (significantly better than the placebo by a factor 2.6).



Illustrative pictures generated by AI of the skin elasticity

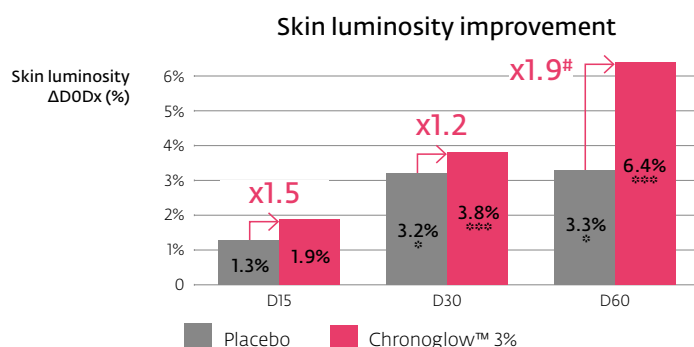


Efficacy

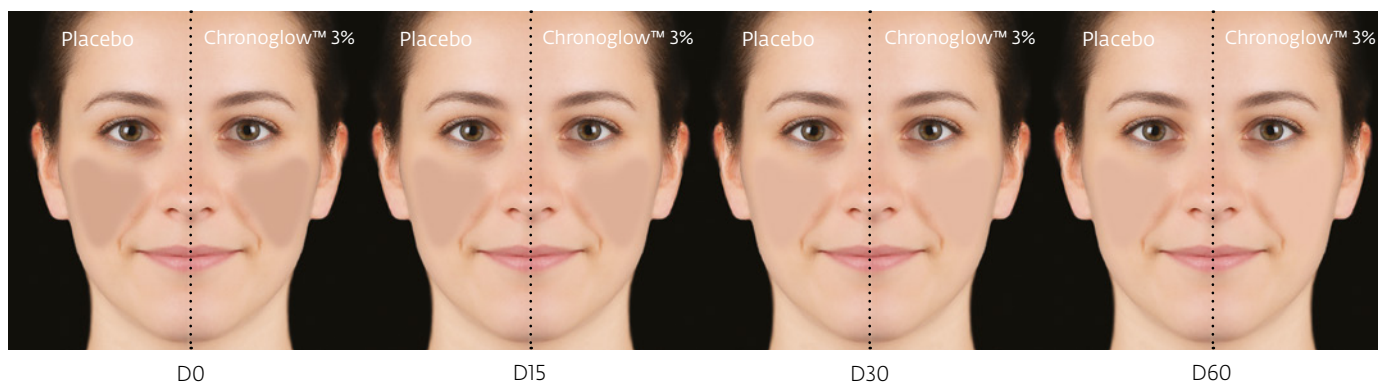
2. Skin tone enhancement - Luminosity

Skin luminosity (L^*) was determined using a spectrophotometer. This parameter is linked to the skin colour brightness.

Results: Chronoglow™ significantly improves skin luminosity, already after 4 weeks of use (by up to +3.8%). This positive impact on the skin luminosity is increasing during the whole duration of the clinical test, with an increase up to +6.4% after 2 months (significantly better than the placebo, by a factor 1.9).



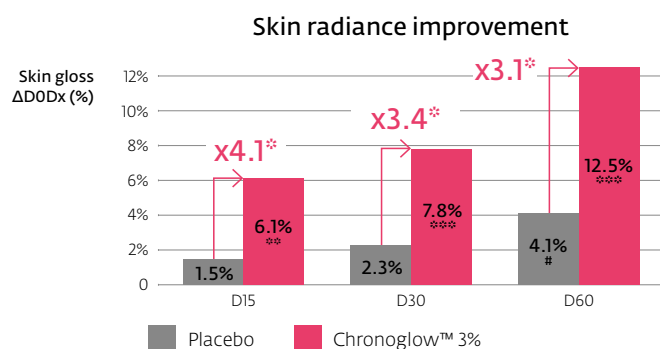
Illustrative pictures generated by AI of the skin luminosity



3. Skin tone enhancement - Radiance

Skin radiance was determined using a spectrophotometer by monitoring the gloss value. This parameter is linked to the skin shininess perception.

Results: Chronoglow™ significantly improves skin radiance, already after 2 weeks of use (by up to +6.1%, more than 4 times better than the placebo). This positive impact on the skin radiance is increasing during the whole duration of the clinical test, with an increase up to +12.5% after 2 months (significantly better than the placebo by a factor 3.1).



Illustrative pictures generated by AI of the skin radiance



Summary



Technical information

INCI:	Glycerin (and) Water (and) Haberlea Rhodopensis Leaf Extract
Origin:	Green fractionation
Preservation:	Preservative free
Appearance:	Pale yellow liquid
Solubility:	Water soluble
Dosage:	1-3%
Processing:	Can be added at the end of the formulation process under stirring or homogenising or can be heated for a short time with the water phase of a formulation. Formulate at pH between 5.5 and 6.5 and temperature below 40°C

Claims

Claims:	Epigenetics control of skin rejuvenation, restructuring, radiance enhancing, firming, protecting from environmental stress.
Applications:	Anti-ageing skin care products, mature skin care, face/neck/body sculpting, eye contour, sun/after sun care, hand creams, skin radiance products, brightening products (in combination with other actives).

Givaudan Active Beauty Sales Offices

Europe

Givaudan France SAS
19-23 rue de la Voie des Bans
FR-95100 Argenteuil (France)

Givaudan UK Ltd
Magna House
76-80 Church Street
Staines, TW18 4XR (United Kingdom)

Naturex SA
250 rue Pierre Bayle - BP 81218
84911 Avignon Cedex 9 (France)

Asia Pacific

Givaudan Singapore Pte Ltd
1 Pioneer Turn
627576 Singapore (Singapore)

Givaudan Shanghai Ltd
298 Li Shi Zhen Road
Pudong Zhang Jiang High Tech Park
201203 Shanghai (China)

Latin America

Givaudan do Brasil Ltda
Av. Eng^a Billings - 2185, Edifício 31, 1^a Andar - Jaguaré
05321-010 São Paulo - SP (Brazil)

North America

Givaudan Fragrances Corp.
40 W - 57th Street - Floor 17
NY 10019 - New York (United States)

global.cosmetic@givaudan.com

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