

anti-photo aging • global cell protection • wrinkle reducing • full spectrum UV, IR and visible light protection • anti-itching • membrane stabilizing • age prevention • DNA protection • long-term moisturization (7days) • skin barrier repairing • anti-inflammatory

Ectoin®

ULTIMATE PROTECTION

NATURE'S MOST SOPHISTICATED
PROTECTION STRATEGY – CONCENTRATED
IN A PURE COSMETIC ACTIVE INGREDIENT

Ectoin® is a very safe, 100% natural and multifunctional cosmetic active ingredient with global cell protection and outstanding anti-aging properties. Various in-vivo and in-vitro studies as well as clinical trials underline the extraordinary activity of Ectoin®.

Ectoin® was discovered in 1985 as the self-defense and survival substance of microorganisms living in a salt lake in Wadi El Natrun (Egyptian desert). The amino acid derivate belongs to the group of Extremolytes. Extremolytes are small stress-protection molecules, which protect extremophilic microorganisms and plants against the lethal and extreme conditions of their habitats like salt lakes, hot springs, permanent ice or deserts.



The Dead Sea: habitat for extremophilic microorganisms

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ECTOIN® – ULTIMATE PROTECTION

Ectoin®: cell protection designed by nature

Salt lakes and deserts are the most hostile environments on earth. It is even more astonishing that under these extreme conditions life is possible since millions of years.

Highly adapted bacteria, so called extremophilic microorganisms defy dryness and extreme changes in temperature and grow well. Responsible for this property is a substance called Ectoin®. It protects these small living beings from harmful environmental influences.

The properties of Ectoin® have been investigated in detail by numerous independent scientific research groups and universities in Germany and worldwide. The fascinating natural protection mechanism of Ectoin® is used in the health care, life science and personal care industry.

Small molecule – big effects

Ectoin® is a strongly kosmotropic substance. The small amino acid derivate binds the water molecules of its surroundings and creates the so called "Ectoin® Hydro Complex" (figure 1). These complexes again surround cells, enzymes, proteins and other biomolecules by forming protective, nourishing and stabilizing hydration shells around them (figure 2).

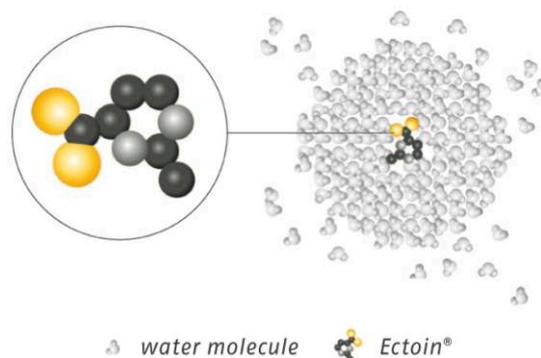


Figure 1: Ectoin® Hydro Complex

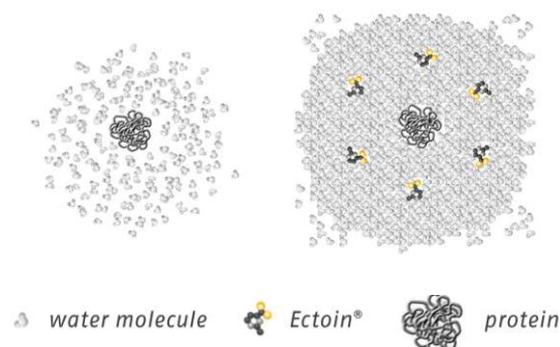


Figure 2: Protection shell around protein

ECTOIN® – ULTIMATE PROTECTION

The Ectoin® protection mechanism is very simple but also very effective: due to the protection shells, stress factors cannot harm the cells any more. Cell damage is prevented (figure 3).

This is how Ectoin® protects skin, mucosa but also hair follicles or lung tissue from damage by all sorts of stress-factors like UV-radiation, pollution particles, allergens, heat and cold, dryness or chemical stress.

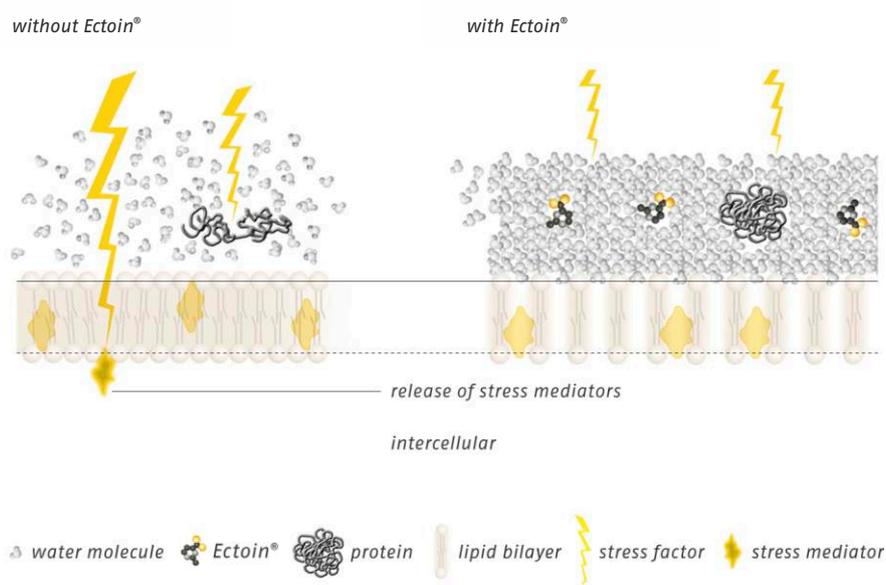


Figure 4: Surface of cell membrane (extracellular space) with and without Ectoin®

Ectoin® has a remarkably positive impact on cells and macromolecules. Various tests clearly displayed the most powerful stabilizing properties for Ectoin® on the integrity of proteins, nucleic acids, biomembranes and whole cells compared to other solutes. By stabilizing for example proteins like collagen or elastin, Ectoin® protects them from unfolding and the consequent damage and denaturation.

Ectoin®: protection from damage and boost of cell functions

The remarkable stabilizing and protective characteristics of Ectoin® lead to visible and long-term anti-aging results for our skin. Clinical studies have shown a long-lasting enhancement of the general skin condition, like the increase of skin elasticity, reduction of wrinkle volume or skin roughness. By stabilizing the skin barrier and restoring and regulating the moisture content of the skin, Ectoin® reduces the TEWL, increases the hydration level of the skin and preserves the skin moisture for 7 days without any re-application!

Furthermore Ectoin® calms and soothes irritated and damaged skin. The skin's regeneration process is significantly increased. Because of its excellent anti-inflammatory and anti-itching properties, Ectoin® is even used for the treatment of atopic dermatitis (neurodermatitis) or allergic skin conditions in health care products (medical devices; OTC products).

EFFICACY – IN VIVO

The natural stress-protection molecule Ectoin® has become available as a protective active ingredient against skin aging. By building up a strong protection layer in the skin, the so called „Ectoin® Hydro Complex“, Ectoin® protects the skin from extrinsic and intrinsic factors of skin aging and cell damage.

At the same time, Ectoin® increases and restores cell functions, which are responsible for the skin's health and beauty. This global protection and stabilizing activity leads to a long-lasting and visible preservation and restoration of youthful and healthy skin.

» Reduction of wrinkle volume (clinical study)

Clinical Trial: test protocol

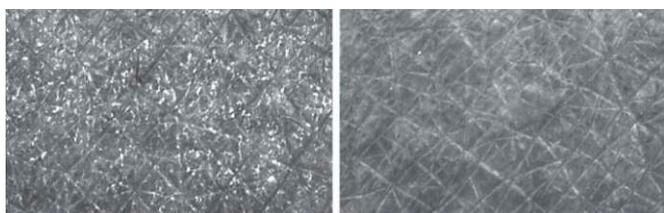
- double-blind, intra-individual, placebo-controlled study
- 24 female volunteers (30–60 years old) with normal, dry and sensitive skin using cream containing 2% Ectoin® 2x/day for 28 days and placebo cream
- skin surface measurement by SELS method, Visioscan at day 0 and day 28

Results

After 4 weeks of treatment with Ectoin® a visible and significant reduction of the skin micro relief was observed. Deep structures of the skin were smoothed. The skin looked healthier and rejuvenated. 100% of the subjects demonstrated a significant effect.¹

23% reduction of wrinkle volume after 4 weeks.¹

Visualization of the smoothing effect by Visioscan



3D representation of wrinkle depth by SELS method

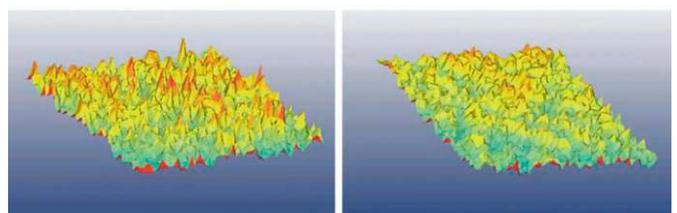


Figure 4: Skin surface measurements by the SELS method (Visioscan) before (left) and after (right) 4 weeks of treatment with a formulation containing 2% Ectoin®.

Source: Heinrich et al., 2007.

» Skin elasticity improvement (clinical study)

Clinical Trial: test protocol

- double-blind, intra-individual, vehicle-controlled application test
- 24 female volunteers (30–60 years old) with normal, dry and sensitive skin using cream containing 2% Ectoin® and placebo 2x/day for 28 days
- visco-elasticity & biologic elasticity measurement with Cutometer® (in AU) at day 0 and day 28

Results

After 28 days of application Ectoin® increased the skin elasticity by 82,4 % compared to the untreated control. Only the Ectoin® treated skin showed a significant improvement of visco elasticity and biologic elasticity.¹

In vivo assessment – 24 test persons, 28 days, Ectoin 2 %

Visco-elasticity	untreated control compared to
Ectoin®	+ 82,4%
Vehicle	+ 38,5%

Table 1: Elasticity measurements (in AU) before and after 4-week treatment with placebo and Ectoin® versus untreated control.

» Improvement of scaling and roughness (clinical study)

Clinical Trial: test protocol

- double-blind, intra-individual, vehicle-controlled application test
- 24 women (30–60 years), normal, dry & sensitive skin cream with 2% Ectoin®, placebo 2x/day for 28 days
- skin roughness and scaling measured with Visioscan®

Results

Skin scaling and roughness were significantly decreased after 28 days of treatment with 2% Ectoin®.¹

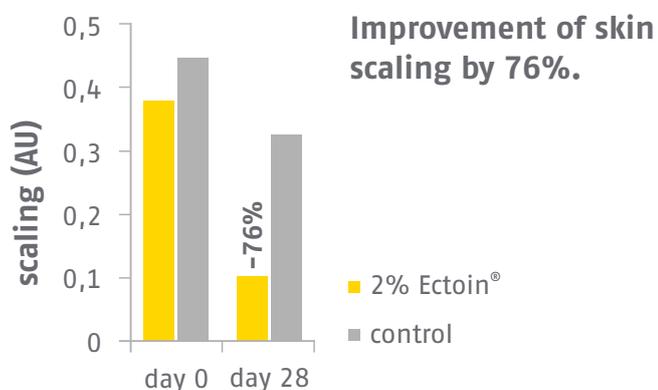


Figure 5: Skin surface parameter scaling by the SELS method (Visioscan) before and after 4-week treatment with Ectoin®.

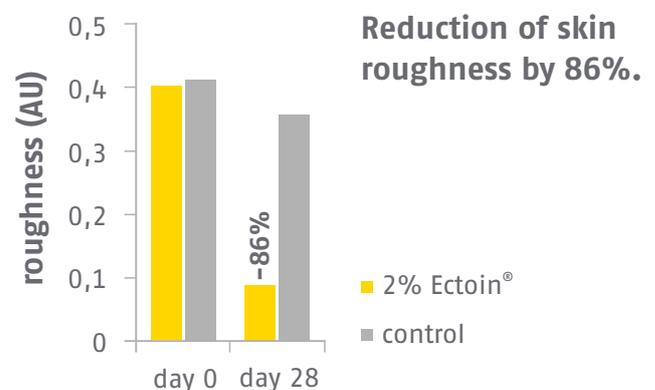


Figure 6: Skin surface parameter roughness by the SELS method (Visioscan) before and after 4-week treatment with Ectoin®.

» Protection of Langerhans cells (in-vivo study)

Ectoin® is proven to 100% protect epidermal immune cells called „Langerhans cells“ from damage. In addition to the skin’s antioxidant defense, these cells help to protect the skin by recognizing antigens and inducing the antibody defense responses.

Langerhans cells are sensitive to external stress factors. A decreasing number of Langerhans cells is a sign of skin damage and aging.

An in-vivo study impressively demonstrated, how Ectoin® prevents aging and skin damage by protecting the Langerhans cells from UV-induced damage and reduction.²

Clinical trial: test protocol

- in-vivo study
- treatment twice daily with 0, 0.3% or 0.5% Ectoin® for 14 days
- UV-stress (1.5 MED) of human forearm
- skin preparation and histological proof of Langerhans cells

Results

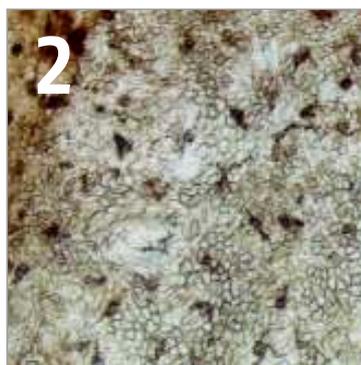
Ectoin® protects the number of Langerhans cells in UV-irradiated human skin and thus reduces UV-induced cellular immune-suppression.²

Ectoin® prevents photo aging by protecting the Langerhans cells from UV-induced damage. 0,5% Ectoin® was capable to protect 100% of the Langerhans cells. UV-stress did not damage the Langerhans cells in the Ectoin® treated areas at all!²

Compare pictures 1 with 4!



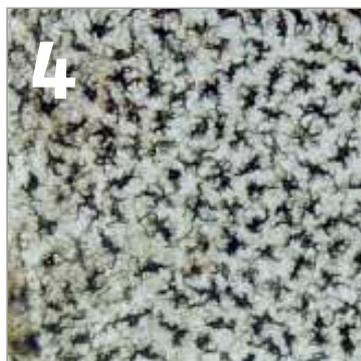
untreated / un-irradiated control



untreated / UV-irradiated



placebo-treated / UV-irradiated



Ectoin® treated / UV-irradiated

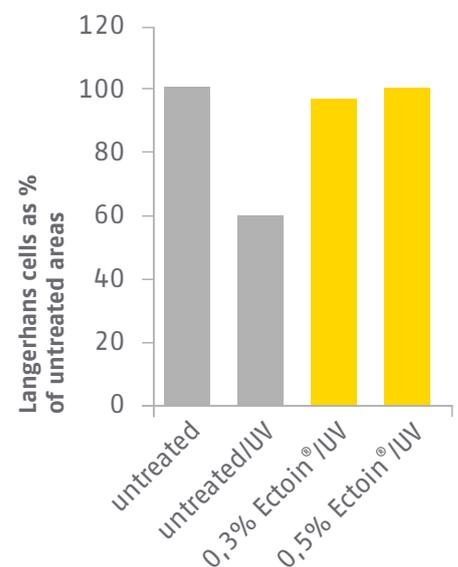


Figure 7: Amount of vital Langerhans cells in % after UV irradiation on untreated and pre-treated skin with 0,3% and 0,5% Ectoin®.

Figure 8: Human epidermis under the microscope. The Langerhans cells have been visualized using ATPase staining. The photographs were taken 48h after irradiation of the skin with 1.5MED. Source: Bünger et al., 2001.

» Long-lasting moisturization ("7days") (in-vivo study)

Clinical trial: test protocol

- double blind, placebo-controlled application test
- 5 female volunteers with normal to dry skin
- formulation with 0%, 0.5% and 1% Ectoin®, applied twice daily for 12 days on forearm
- skin hydration was measured from day 8 to day 12
- on day 12, application of Ectoin® was stopped for 7 days
- at day 19: detecting the skin hydration again for final result



Results

During the test, the hydration level in the skin increased up to 200% compared to the placebo treated skin. Although the topical application was stopped on day 12 for the following 7 days, the hydration status was preserved during these 7 days, underlining a significant long-term moisturizing effect of Ectoin®.³

The results of this placebo-controlled study demonstrate the outstanding moisturization properties of Ectoin®: After 8 days of application, the hydration level increased significantly up to 200% and remained constant until the end of the study period (day 19).³

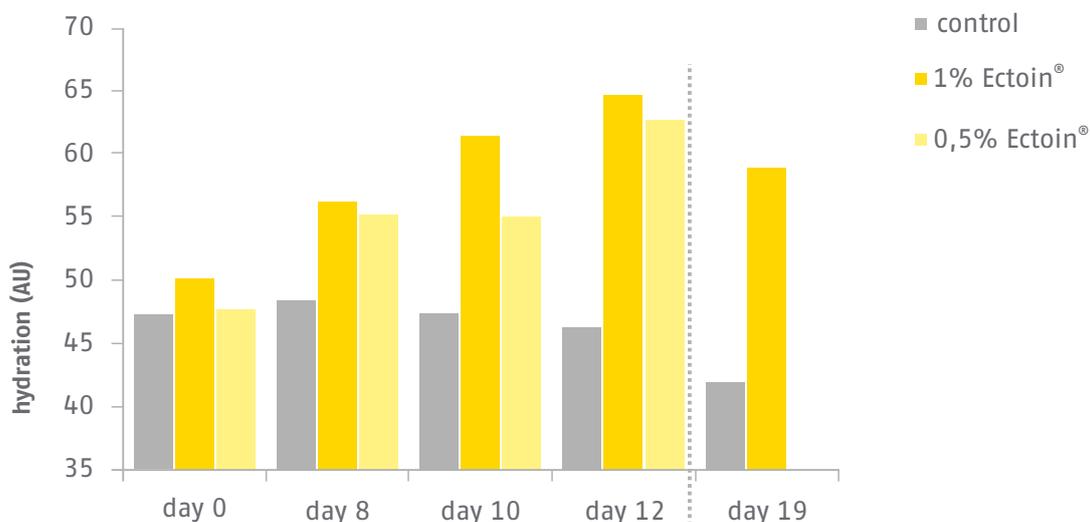


Figure 9: Long-term moisturizing effect with Ectoin®. The skin hydration status was determined with corneometry. The application was stopped on day 12 for another 7 days. The study was finalized on day 19 with a last measurement of the skin hydration status. Source: Graf et al., 2008.

Increase of skin hydration up to 200% and preservation of hydration status for 7 days!

The skin moisture can be maintained for a longer period of time with Ectoin® compared to placebo treated areas – even in atopic skin. Furthermore the application of emulsions containing Ectoin® always led to remarkable reductions of the TEWL. Skin, treated with Ectoin® becomes less vulnerable to damage by stress factors like surfactants and the consequent loss of water. Ectoin® restores the water content in the skin and protects the skin from rapid dehydration.

»» Reduction of TEWL and skin dryness (in-vivo study)

One of the major goals of cosmetics is still the protection of the skin against stress factors that lead to dehydration. Dry air, particularly during periods of freezing or hot weather and air conditioning tends to dry out the skin considerably.

The stratum corneum provides a barrier to the evaporation of water from the epidermis. Daily, our skin is exposed to a variety of environmental assaults, which cause damage to this barrier and increase the rate of water loss from the skin, leading to skin dryness. In addition to dryness the cumulative effect of external factors leads to accelerated skin aging.

Skin with a suboptimal or damaged barrier shows increased transepidermal water loss (TEWL) and diminished moisturization.

The influence of Ectoin® on the TEWL was investigated in several placebo controlled in-vivo studies. Furthermore also the protective effects of Ectoin® on skin moisture were tested.

Clinical trial: test protocol

- double blind placebo controlled application test
- 5 volunteers with normal to dry skin
- formulation with 0% and 2% Ectoin®, applied twice daily for 7 days
- moisture content of the skin is determined before application and after 1 week (4 hours after final application)
- silica gel is applied under occlusion for 2 hours to cause dehydration; on removal of the silica gel the skin moisture content is determined after 10min, 2 hours, 4 hours and 24 hours

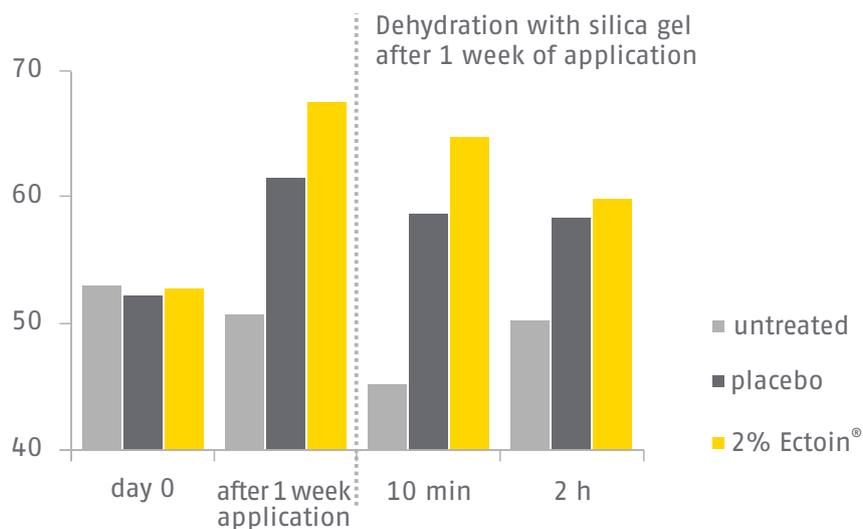


Figure 10: In vivo determination of skin moisture after treatment with Ectoin® and subsequent dehydration with silica gel. The moisture content of the skin is determined at day 0 and after 1 week of application. Determination of skin moisture after silica gel is applied for 2 hours after 10 min and after 2 hours. Source: Graf et al., 2008.

Results

Ectoin® protects the skin against dehydration. In addition to this protection, Ectoin® also produces a higher moisture content than the placebo formulation that already contains 3% glycerol. The results also show, that even after 24 hours Ectoin® maintains a considerably greater degree of skin moisture than untreated or placebo-treated skin. Ectoin® even protects skin against rapid dehydration after direct application of hygroscopic silica gel. Skin moisture can be maintained for a longer period of time with Ectoin®.³

EFFICACY – IN VITRO

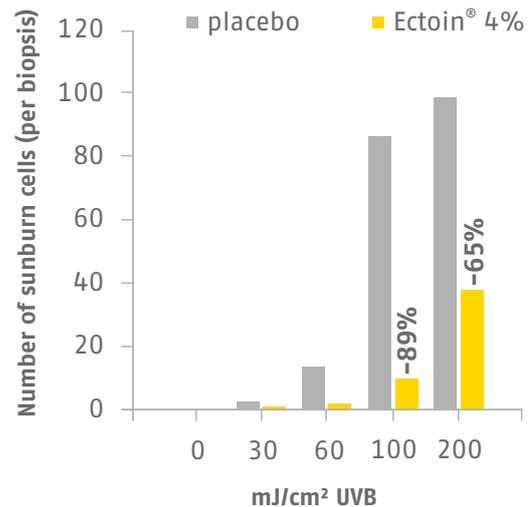
»» Reduction of sunburn cells (skin model)

The existence of sunburn cells in the epidermis is a hallmark of UV-induced skin damage. Sunburn cells are keratinocytes undergoing apoptosis (controlled cell death) after they have received a UV dose, that severely damaged their DNA or other chromophores and repair was no longer possible.

Test protocol:

- organotypic skin model
- 24 hours pre-treatment with 4% Ectoin® or placebo
- UV-radiation of organotypic skin (Skinethic®)
- histological proof of sunburn cells

Figure 11: Number of sunburn cells per biopsy after UVB irradiation of the organotypic skin. The skin was pre-treated with a formulation containing 4% Ectoin® or placebo.



Results

Ectoin® significantly reduces the number of sunburn cells. It protects the skin cells against harmful effects and damages induced by sun exposure.²

The number of sunburn cells was reduced by up to 89%.

»» Heat protection – boost of the skin’s self defense

Human skin cells produce heat-shock proteins (HSP) under external stress like UV-radiation or other physical and chemical stress factors. Heat-shock proteins recognize, stabilize and metabolize damaged proteins in the skin. They are an important part of the skin’s self defense system.

Test protocol:

- human keratinocytes pre-treated with 1% Ectoin®
- expose to heat-shock for 60 minutes
- produced stress proteins (HSP 72/72) marked with fluorescent antibodies

Results

The pre-treatment of keratinocytes with Ectoin® resulted in a more rapid supply of heat-shock proteins. Ectoin® treated skin cells reacted 2 to 3 times faster to heat stress than untreated cells.² Ectoin® boosts the supply of heat-shock proteins (HSP) in skin cells.

The protective properties of Ectoin® regarding the whole sun spectrum were demonstrated in various studies.

Ectoin® prevents the Langerhans cells from being damaged or even destroyed. It also reduces the number of sunburn cells developed as a result of the human skin being exposed to UVB-radiation.

Ectoin® does not act like a filter. It does not absorb the UV radiation but protects the skin by surrounding the skin cells and all other components of our skin with protection-shells. This protection mechanism is even capable to shield the skin from the destructive effects of damaging UVA, UVB, IR and visible light irradiation.

» UVA, IR and visible light protection

The sun radiation consists of a wide spectrum of radiation. In the last years, "visible light" was discovered to be damaging for the skin tissue as well. Visible light also induces DNA damage as it produces DNA single strand breaks, sister chromatid exchanges and intracellular reactive oxygen species (ROS) which therefore exert an indirect genotoxic effect via oxidative DNA lesions. Because the activity of antioxidant enzymes and the levels of non-enzymatic antioxidants decline with age, Ectoin® can prevent such oxidative damage in skin. Most commercialized sunscreens afford effectiveness against UVB and UVA but do not give significant protection against visible radiations. The photo protective properties of Ectoin® against the combination of visible light and UVA-radiation and visible only light were tested on human dermal keratinocytes.

Test protocol:

- pre-treatment of keratinocytes with Ectoin® (0,025, 0,05 and 0,1mM)
- keratinocytes were irradiated by combined light (UVA/visible) and visible light (dose of 15 J/cm²)

Results

All tested concentrations of Ectoin® showed a highly significant level of photo protection against the combination of UVA/visible light and the irradiation with visible light only.

Ectoin® completes sunscreen and day care formulations for a full spectrum protection against UVA-radiation and visible light damage.⁴

Level of photo protection: 92,7% (visible light) and 68,9% (UVA/visible).⁴

» Block of UVA-induced DNA damage

Repetitive UVA-radiation is known to cause time- and dose dependent mutations and damage of mitochondrial DNA in skin cells, which consequently leads to premature photo aging.

Test protocol:

- human dermal fibroblasts were pretreated with 1mM Ectoin® over 24h
- fibroblasts were exposed 3 times per day to 8 J/cm² on 4 consecutive days over 3 weeks
- the mutations of the mitochondrial DNA were measured with polymerase chain reaction

Results

Pre-treatment with Ectoin® was sufficient to completely inhibit UVA-radiation induced mitochondrial DNA mutation!^{2,5} The cells showed no degeneration or damage at all.

» Inhibition of UVA-induced skin damage

Test protocol:

- human dermal keratinocytes, 24 hours pre-incubation of keratinocytes with 1 mM Ectoin®
- irradiation with 30 J/cm² UVA for 1 hour
- determination of second messenger release, AP-2 activation and ICAM-1 expression

Results

Ectoin® completely inhibits the UVA-induced second messenger release, the activation of transcription factor AP-2 and the expression of ICAM-1!^{5,6}

The measured parameters show, that Ectoin® inhibits the UVA-induced damaging cascade and consequently the cell aging process in keratinocytes and fibroblasts.⁵

Ectoin® counteracts the effects of UVA-induced and accelerated skin aging at different cell levels.

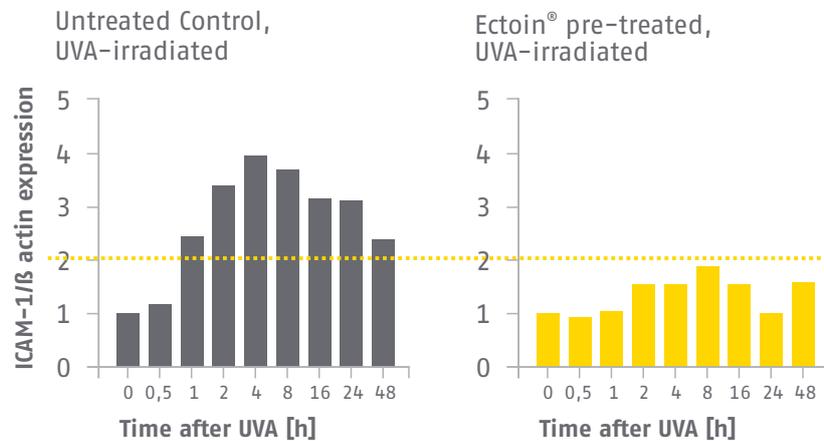


Figure 12: UVA induced ICAM-1 mRNA expression in comparison with the inhibition of these expressions by Ectoin® pre-treatment.

UVA-radiation is the major component of the UV solar spectrum that reaches the earth. Next to other mechanisms, UVA-radiation initiates the gen-regulatory mechanism in human keratinocytes that are responsible for the damage caused by such radiation. It has been shown that the formation of singlet oxygen in the membrane of UVA-radiated keratinocytes releases ceramides that, as second messengers, initiate an UVA-induced activation of the transcription factor AP-2 and expression of pro-inflammatory genes like ICAM-1. In addition to UVA-induced ICAM-1 expression, matrix metalloproteinases are activated and cause wrinkles by degradation of the extracellular matrix proteins (e.g. elastin and collagen).^{5,6,7}

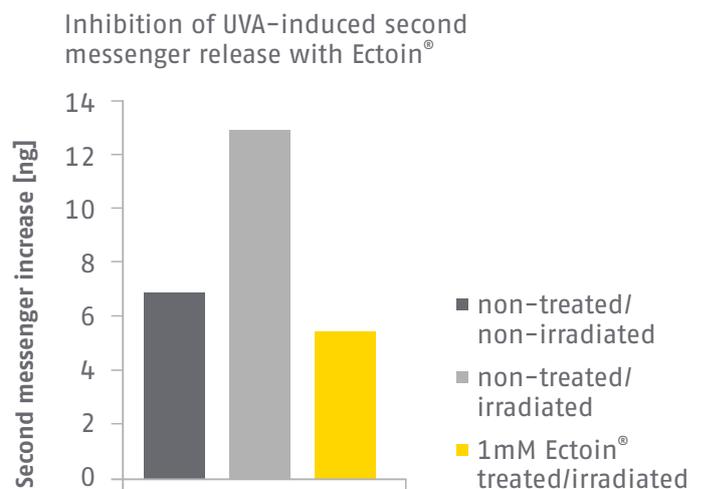


Figure 13: UVA-induced second messenger release in untreated and pre-treated cells with Ectoin®.

HAIR CARE – EFFICACY

Oxidative stress followed by DNA damage and changes in mitochondrial functionality have been discussed to be etiological for aging processes in human beings. Additionally, it has been shown that also hair follicles, similar to other human tissues, exhibit age related changes on the molecular level such as an altered gene expression of structural proteins. Further studies showed a link between cellular aging parameters in the hair follicle and oxidative stress. In vivo, in vitro, and in situ data identified H₂O₂-induced oxidative damage in the entire human hair follicle, inclusive of the hair shaft, as a key element in hair graying, which does not exclusively affect follicle melanocytes.⁸

»» Anti-aging for hair follicles

The effects of Ectoin® on the follicular aging process were investigated in a study with follicular outer root sheath (ORS) keratinocytes and on reconstructed hair follicle models. The study showed, how Ectoin® counteracts typical aging characteristics in follicular cells. The cells were subjected to oxidative stress (H₂O₂).⁹

Test protocol:

- follicular outer root sheath (ORS) keratinocytes and reconstructed hair follicle models were stressed with sub-lethal doses of hydrogen peroxide with and without pre-treatment with Ectoin®
- measured and determined parameters: expression of structural proteins and mitochondria relevant genes, amount of produced reactive oxygen species (ROS) and the degree of DNA damage (determined by COMET assay)

DNA damage in ORS keratinocytes after treatment with Ectoin® followed by hydrogen peroxide stress

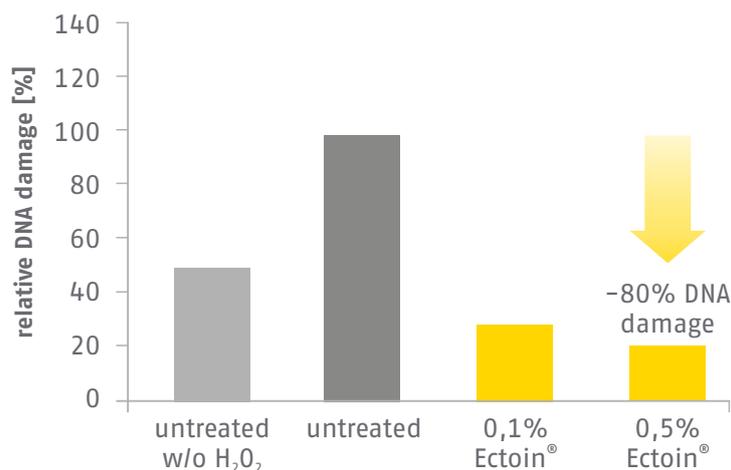


Figure 14: DNA protection in ORS keratinocytes after 24h treatment with Ectoin® followed by challenging the cultures with H₂O₂ (100 µM, 20 min). Values calculated related to %tail DNA. Source: Giesen et al., 2013.

Gene expression of hair keratins in reconstructed hair follicle models after treatment with Ectoin® followed by hydrogen peroxide stress

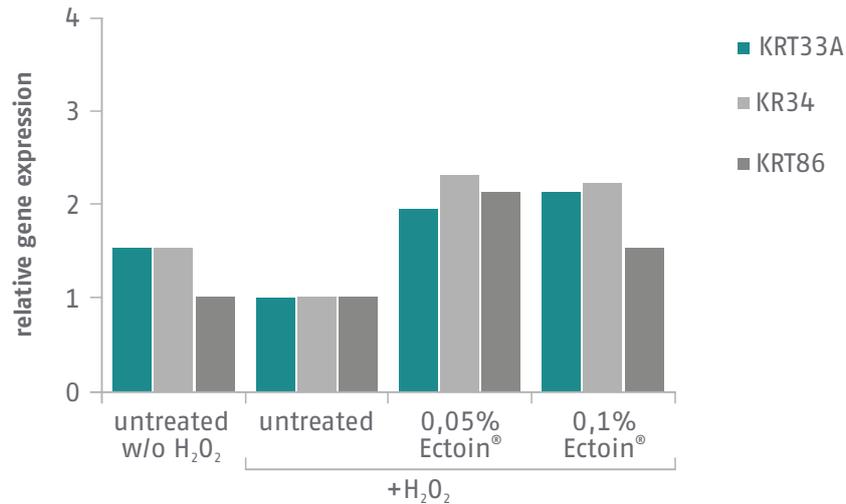


Figure 15: Keratin gene expression after 6h treatment with Ectoin® followed by challenging the cultures with H₂O₂ (100 µM, 20 min). Values calculated related to the H₂O₂ control. Source: Giesen et al., 2013.

ROS production in ORS keratinocytes after treatment with Ectoin® followed by hydrogen peroxide stress

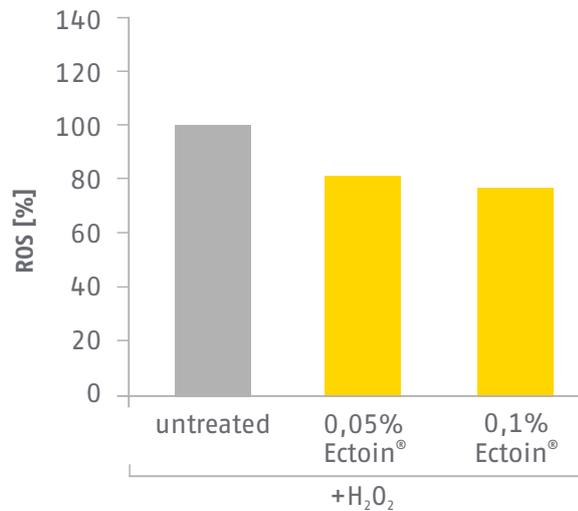


Figure 16: ROS reduction in ORS keratinocytes after 24h treatment with Ectoin® followed by challenging the cultures with H₂O₂ (0,03 mM, 10 min). Source: Giesen et al., 2013.

Results

Ectoin® exhibits a significant restoration of essential cellular parameters under oxidative stress and also a significant reduction of DNA damage up to 80%.⁹

Ectoin® protects the cellular functions, mitochondrial DNA and the keratin synthesis from oxidative stress.⁹

Ectoin® is able to antagonize the follicular aging processes.⁹

»» References

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Product description

Applications

- » skin care (face,body)
- » sun care
- » oral care
- » baby care
- » hair care
- » color cosmetics

explicitly suggested for ultra sensitive and allergic skin

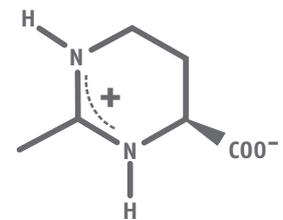
Formulation guidelines

- » level of use 0,3–2%
- » no incompatibility with other cosmetic active substances
- » can be added directly to aqueous phase prior to emulsification

Appearance	white crystalline powder, odorless
INCI	Ectoin
Purity	≥ 96% (≤ 4% Hydroxyectoin)
Solubility	excellent solubility in water (390g/l), Glycerol, Propylene glycol
Chemical name	(4S)-2-methyl-1,4,5,6-tetra- hydroypyrimidine-4-carboxylic acid
Molecular formula	C ₆ H ₁₀ N ₂ O ₂
CAS	96 702-03-3
Molar mass	142.16 g/mol
Production process	biotechnological fermentation / 100% natural origin
Shelf life	5 years
Stability	pH 1–9, temperature up to 80°C, light and oxygen stable
Storage conditions	dry place, temperature from 15° – 25°
Pack sizes	1kg, 5kg, 25kg

100% safe
GMO-free
China compliant
non allergenic
Ecocert
preservative free

Ectoin[®]
ULTIMATE PROTECTION



Ectoin[®] is a patented active ingredient.

bitop

Extremolytes for Life

bitop AG
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