DN-Age™
by Beauty Creations

Global skin rejuvenation
Among environmental factors, the main origin of DNA damages in human skin is the oxidative stress due to UV radiations.

Our goal: to prevent these damages by protecting the 2 types of DNA from the whole UV spectrum:

1) Protection of the nuclear DNA against UVB
At nuclear level, UVB mainly induce DNA fragmentation and thymine dimers formation, leading to strong metabolism modifications (accelerated skin photo-aging) and/or disorganized multiplications (skin cancers).

2) Protection of the mitochondrial DNA against UVA
New studies show that a repetitive UVA exposure is responsible for the loss of a fragment from the mitochondrial DNA (mt DNA) in human skin cells, so called “the common deletion”.

This generation of mtDNA deletions in dermal fibroblasts is thought to disrupt mitochondria functioning and impair proper energy synthesis, accelerating skin aging. It has been demonstrated that dermis of photo-aged skin contains a significantly larger amount of mtDNA deletions in comparison with dermis of normal skin.

Against these aggressions, in a normal state, cells can rely on their own capacity to prevent and to repair damages due to UV, in order to keep their full integrity.

Unfortunately, due to some factors such as aging or after a strong saturated stress, this natural repairing system can not be sufficient. In this case, it is necessary to help the cells to prevent UV damages and to support their endogenous repair system. The answer has been found in Nature: the protective molecule K3OS (Kaempferol-3-O-Sophoroside), naturally occuring in the plant Senna alata.

From this original vegetal source, Beauty Creations has developed a new active ingredient targeting a complete DNA protection:
- by preventing UV induced DNA damages on both nuclear and mitochondrial DNA,
- by acting on the whole UV spectrum even under the MED (minimum erythema dose), source of insidious cell damages,
- by supporting the natural repairing capacity of the cell when necessary,
- by preventing microrelief alteration due to UV exposure with an effect readily perceivable by the end user.

DN-Age®, DNA phyto guardian: clever, self regulated protection.
Definition / Composition

DN-Age® is an extract of the leaves of the candle tree (Senna alata (L.) Roxb) specifically selected for its high amount of K3OS (Kaempferol-3-O-Sophoroside). K3OS is over expressed in the sun-exposed leaves, as a natural protective system of the plant against UV radiations. It is fixed in and around the cell nucleus of the plants. Its main function is to protect the plants against free radicals and DNA damages induced by UV radiations especially at the level of the epidermis of the leaves and of the nucleus of vegetal cells. This protective efficacy extends to the protection of human DNA: K3OS has been identified as the active tracer responsible for DN-Age® efficacy.

Skin benefits

DN-Age® acts on the deep effects of UV radiation on the skin, by reducing the photo-induced damages on DNA at the nuclear and mitochondrial levels, but also by acting on the visible signs of photo-aging such as the prevention of microlief alteration due to sun exposure (demonstrated by a clinical study). This auto regulating protective activity against the chronic harmful effects of the sun helps the cells to keep their “youth”.

Cosmetics use

- Anti-aging sun care products for face and body.
- “Skin self-protect” daily care.
- Specific skin care ranges focusing on photo-aging effects: prevention and protection.

Dosage / Solubility / Mode of incorporation

1. Dose of use for:
   - DN-Age™ LS9547 1 to 3%.
   - DN-Age™ PW LS9827 0.1 to 0.25%.
2. Solubility: soluble in water, insoluble in oils and fats.
3. Mode of incorporation: to be incorporated at a temperature below 60°C during the finishing process or at room temperature for cold formulations. Optimal pH: 4 - 7.

Analytical characteristics

1. Aspect: dark brown opaque liquid with a characteristic odor.
2. Specifications: upon request.

Tolerance

Good.

Efficacy

Test summaries hereafter.

Storage

In its original packaging, at 15 - 25°C.

INCI name

DN-Age™ LS9547 Water (and) Glycerin (and) Cassia Alata Leaf Extract.
DN-Age™ PW LS9827 Maltodextrine (and) Cassia Alata Leaf Extract.
Protective activity on the cell nuclear DNA against UVB damages

**Inhibition of thymine dimers formation** *(immunocytochemistry on keratinocytes)*

**Aim**
Under UVB radiations, even below the MED, there is formation of thymine dimers, which are responsible for strong DNA structure modifications. The rate of formation of thymine dimers in skin is linearly correlated to the UV dose (fig. 1).

The aim of the test is to demonstrate the capacity of DN-Age® to reduce the thymine dimers formation under UVB. The results have been compared with the activity of K3OS, the active tracer of DN-Age®.

**Efficacy tests**
Tests have been selected in order to demonstrate the comprehensive protective activity of DN-Age®:
1) on the cell nuclear DNA against UVB damages,
2) on the mitochondrial DNA against UVA damages.

**Conclusion**
DN-Age® and K3OS significantly reduce the level of thymine dimers induced by UVB. This effect is dose dependant. DN-Age® protects cell nuclear DNA against UVB damages. As K3OS displays the same activity, this demonstrates that the DNA protective activity of K3OS in the plant can be transferred from the vegetal cells to the skin cells.
Inhibition of the DNA fragmentation (comet assays on keratinocytes)

Aim
Next to thymine dimers formation, strand break is a critical nuclear DNA damage induced by UVB. Comet assays allow to demonstrate the capacity of DN-Age® to protect DNA against fragmentation.

Protocol
When submitted to an electric field, DNA fragments tend to move away from the center of the nucleus, generating a «comet shape».

The distance between the 2 inertia centers of the comet reflects the amount of damage.

The higher the value, the more DNA fragments moved away.

Fig. 5 - Schema of the protocol.

Results
2D image analysis

DN-Age® significantly reduces the DNA fragmentation in keratinocytes irradiated by UVB (reduction of «comet shape»).

Fig. 6 - 2D comet assays.

3D image analysis

Fig. 7 - 3D comet assays.

Conclusion
DN-Age® decreases UVB-induced DNA fragmentation. This protective activity against UVB radiations complements the efficacy already evidenced on prevention of thymine dimers formation.

Fig. 8 - Comet assays: 3D quantitative results.
Chronic repetitive exposure to UVA radiation induces mtDNA deletions (missing fragments in circular mtDNA) in human dermal fibroblasts. The main consequences of mtDNA deletions in dermal fibroblasts are improper mitochondria functioning and reduction of cell metabolism, which results in accelerated skin aging.

Aim
Demonstration of the protective efficacy of DN-Age® against UVA-induced mitochondrial DNA deletions.

Culture of primary human dermal fibroblasts
Exposure 3 times / day to sub-lethal doses of UVA (8 J/cm²)
4 consecutive days / week for 1 week or 2 weeks
Assessment of the formation of the common deletion
Semi-quantitative PCR (Polymerase Chain Reaction) technique
Visualization of amplification products in agarose gel stained with ethidium bromide and quantification by phosphorimager analysis

Fig. 9 - Schema of protocol.

Results

Fig. 10 - Rate of the «common deletion» in UVA-stressed fibroblasts: results of 1 typical assay from 5 replicates.

Conclusion
The UVA irradiation (lane B) has induced a high level of «common deletion» in mtDNA (pink arrow), while there is no generation of «common deletion» in the control (lane A). Lane P refers to a batch of mtDNA featured by a defined level of «common deletion».
DN-Age® (lane C) provides a complete protection against UVA-induced mtDNA damage.
**DNA repair: stimulation of gadd45α gene expression - quantification by qRT-PCR**

**Aim**

Gadd45α is involved in the natural DNA repair process of cells. It supports the NER (Nucleotide Excision Repair), reinforcing the repair process of UV-induced DNA damage. We have evaluated the potential of DN-Age® to stimulate Gadd45α and the DNA repair of human epidermal keratinocytes, using a new in-vitro method, quantitative RT-PCR or real time PCR.

This method consists of measuring the amount of messenger RNA (mRNA) encoding for Gadd45α in cultured human keratinocytes.

**Protocol**

Seeding of human epidermal keratinocytes

Incubation for 72-120 hours at 37°C

Exchange of cell culture medium to a defined balanced salt solution with a range of products to be tested

Control K3OS DN-Age®

Incubation for 90 and 180 minutes at 37°C

Recovering of keratinocytes for RNA extraction

qRT-PCR reaction for mRNA of gadd45α and actine gene

**Results**

Fig. 12 - Activity of DN-Age® on Gadd45α gene expression in human keratinocytes.

**Conclusion**

The Gadd445α gene expression rate does not change between 90 and 180 minutes demonstrating that the Gadd445α gene is steadily expressed in culture keratinocytes. 0.1% K3OS and 1% DN-Age® have significantly increased the expression of the Gadd445α gene which encodes for proteins involved in the natural DNA repair process.

By enhancing the Gadd445α gene expression, it is proven that DN-Age® is able to support the natural DNA repair process.
Improvement of skin microrelief (clinical test)

Aim
The detrimental effects of UV exposition are visible at the level of the skin microrelief. We have demonstrated the protective activity against photoaging of a cream containing 3% of DN-Age®: prevention of skin microrelief alterations after UV irradiation.

Protocol
Study on 12 female volunteers aged from 30 to 50 years old with phototype II and IIIA.

![Protocol diagram](image)

Fig. 13 - Schema of protocol: pretreatment during 6 days. Treatment + irradiation during 4 days. Visualization of microrelief alterations after 6 days.

Results

![Results graph](image)

Fig. 14 - Quantitative study of negative replica by confocal microscopy.

Fig. 15 - Study of negative replica by confocal microscopy 6 days after the last irradiation. Fast Fourier transform (FFT).

Conclusion
A good quality microrelief is characterized by high isotropy. DN-Age® allows to prevent the loss of isotropy induced by UV irradiation. The cutaneous microrelief is significantly protected by DN-Age® at 3% in a cream after 4 daily consecutive UV (A + B) irradiations. The aspect of the skin microrelief is totally preserved. This effect can be readily perceived by the end user.

DN-Age®, by targeting a comprehensive protection of cellular DNA, has a good preventive activity against skin photo-aging.

This protective efficacy has been demonstrated at cell level and can be visualized at skin surface.

Complementary tests
Cytoprotection of human keratinocytes against UVB (in-vitro).
After UVB radiations, DN-Age® and K3OS increase the cell survival and reduce the rate of released LDH and PGE2.
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