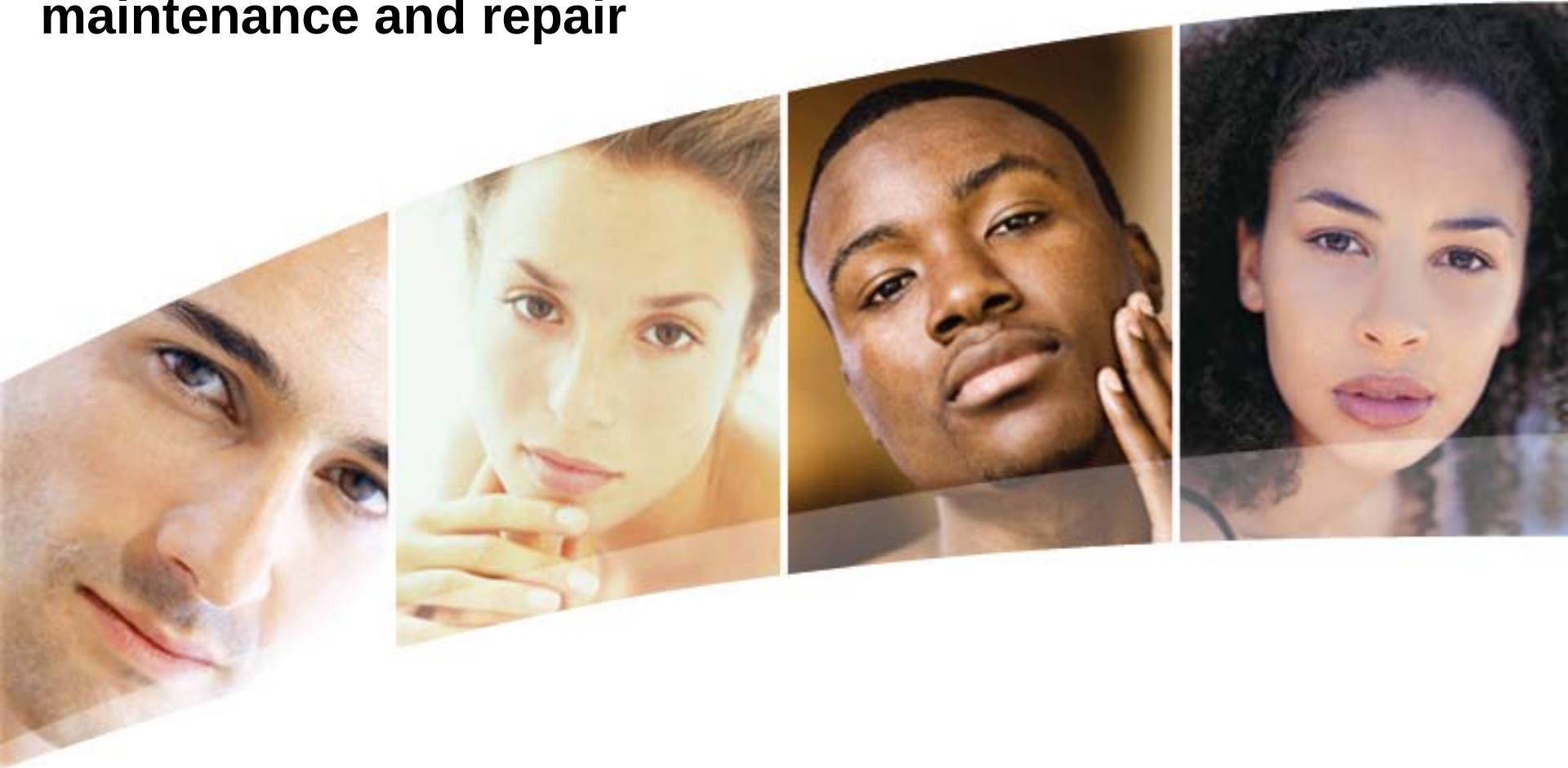


GP₄G SP biofunctional
Helps energize and protect skin from
environmental stresses, to enhance
maintenance and repair

*V*inci^{ence}
Biofunctionals



ASHLAND®

With good chemistry great things happen.™

GP₄G SP biofunctional

Helps energize and protect skin from environmental stresses, to enhance maintenance and repair.

A

Description

B

Energizing
properties

C

Skin
maintenance
and repair

D

Epidermal and
dermal
regeneration

E

Technical
information

GP₄G SP biofunctional



Presentation

Composition

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Properties

GP₄G SP biofunctional



Presentation

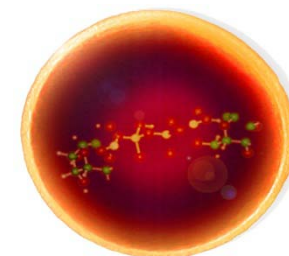
GP₄G SP is a biofunctional ingredient extracted from the plankton *artemia salina*.

GP₄G SP is designed for energizing and regenerating skin care products.

GP₄G SP offers a new way to help boost skin defenses against stress.

It shows **multiple effects on skin maintenance and repair:**

- Helps limit UV-induced DNA damage (*in vitro*)
- Stimulates HSP70 *in vitro*, key proteins in the fight against cellular stress
- Helps boost skin defenses as well as resistance to UV and heat-stress (*ex vivo*)
- Helps limit IL-1 α in UV-stressed cells (*in vitro*)



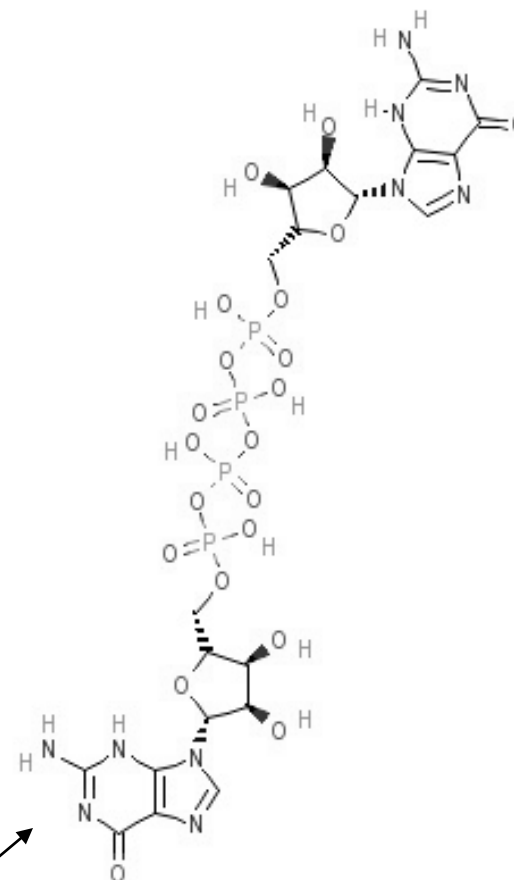
Cyst of artemia salina with GP₄G molecule

GP₄G SP biofunctional



Composition

The originality of GP₄G SP is based on its composition rich in nucleotide energetic messengers, and in particular, in the presence of an unusual nucleotide:



Diguanosine tetraphosphate (GP₄G)

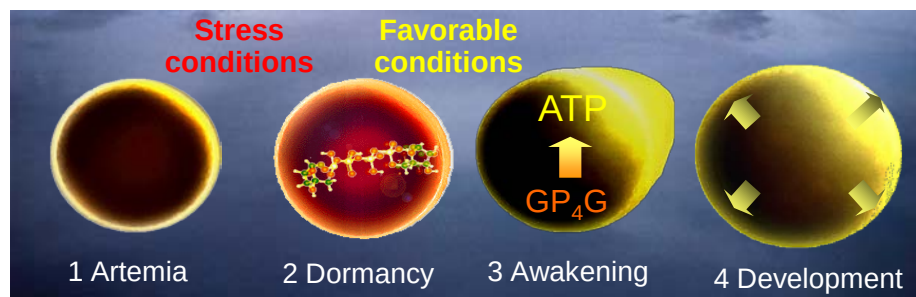
GP₄G SP biofunctional



Origin

1. Artemia is a plankton that lives in hypermineral lakes for several million years.
2. When environmental conditions turn critical (stress), Artemia enters a state of dormancy, encapsulates and accumulates a vital molecule, the Diadanosine Tetraphosphate (GP₄G), a principal source of phosphate bonds with high energetic potential used for DNA protection as well as energy storage.
3. When environmental conditions turn favorable again, Artemia 'awakens', re-hydrates and different metabolic activities take place again. Ultimately, GP₄G is transformed into ATP.
4. GP₄G provides Artemia with energy and protection for its survival and development.

Life cycle of artemia salina plankton



GP₄G SP biofunctional



Properties

Energizing properties

- Helps skin increase cellular cAMP second messenger (*in vitro*)

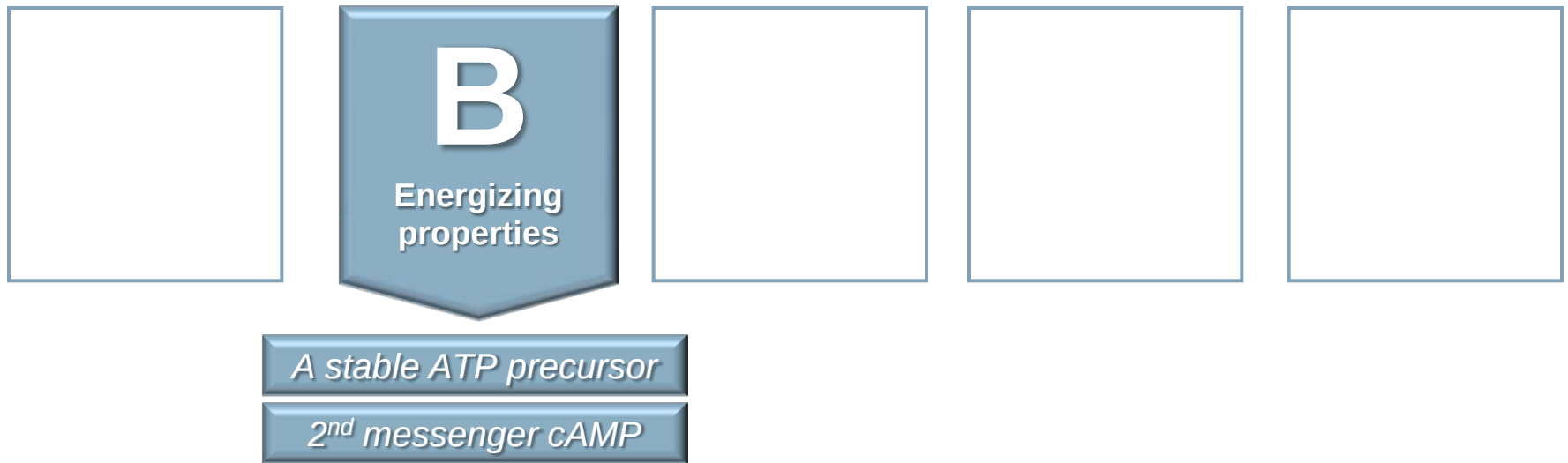
Skin maintenance and repair

- Helps skin limit UV-induced DNA damage (*in vitro*)
- May help skin synthesize Heat Shock Proteins HSP70 (*in vitro*, *ex vivo*)
- Helps skin compensate age-related decrease in Heat Shock Proteins HSP70 (*ex vivo*)

Epidermal and dermal regeneration

- Boosts epidermal keratin and filaggrin expression (*in vitro*)
- Boosts dermal collagen and fibronectin expression (*in vitro*)

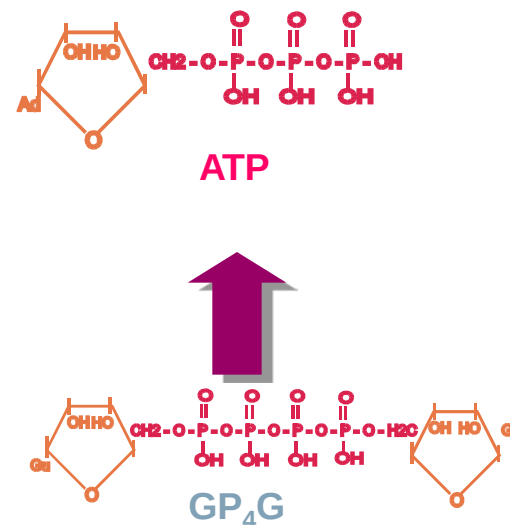
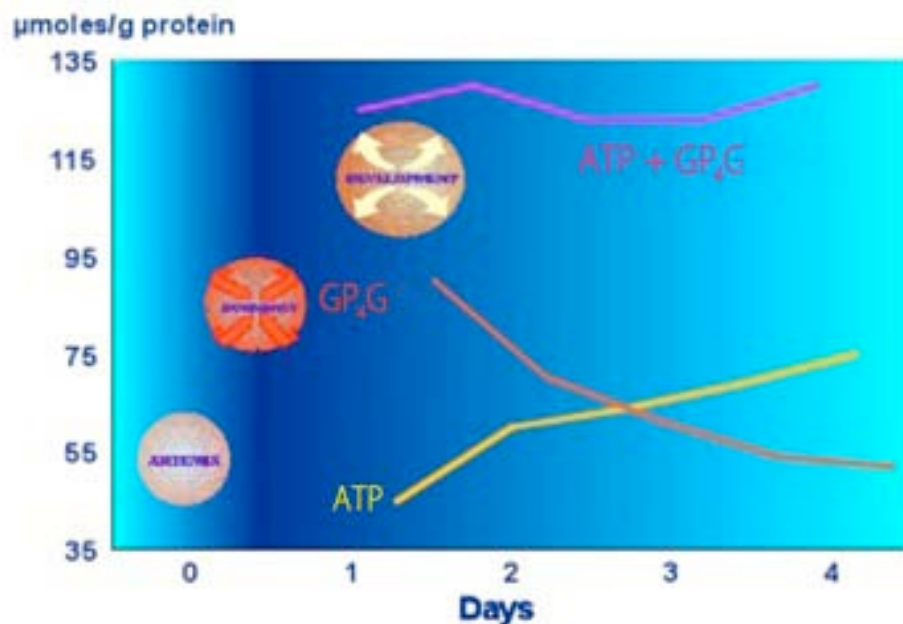
GP₄G SP biofunctional



GP₄G SP biofunctional



Energizing: GP₄G is a stable precursor of ATP



When *Artemia* is awakened by favorable conditions, GP₄G content declines rapidly while a sharp increase in ATP is observed. This is explained by a conversion of GP₄G into ATP and a role of the GP₄G in the awakening of the quiescent cells.

GP₄G SP biofunctional



Energizing: cAMP 2nd messenger

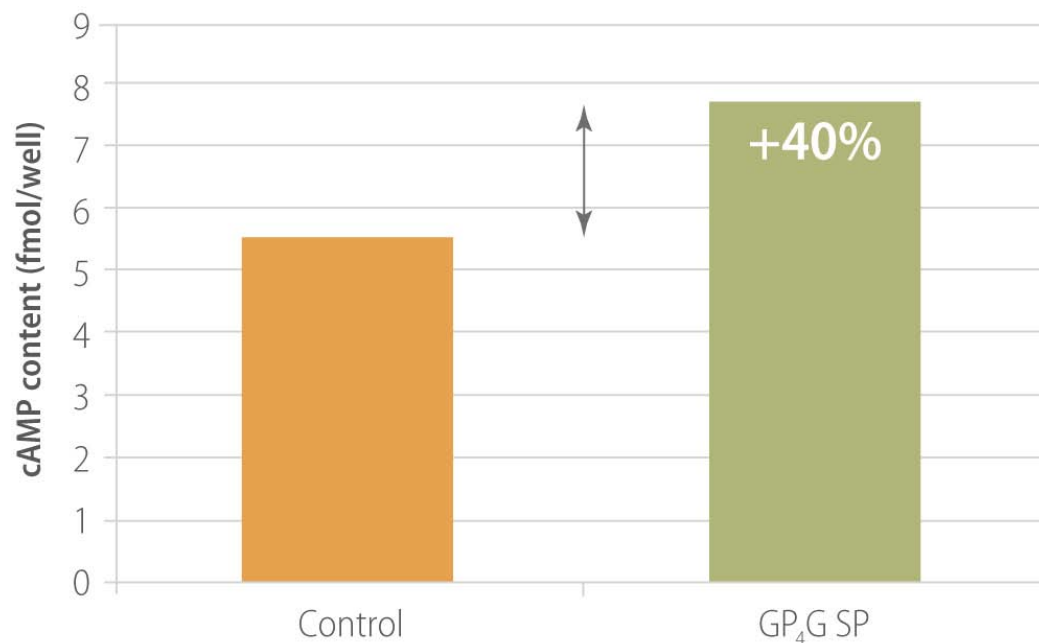
Cells: Human fibroblasts

Use level: Biofunctional at 2%

Evaluation: Immunoenzyme assay

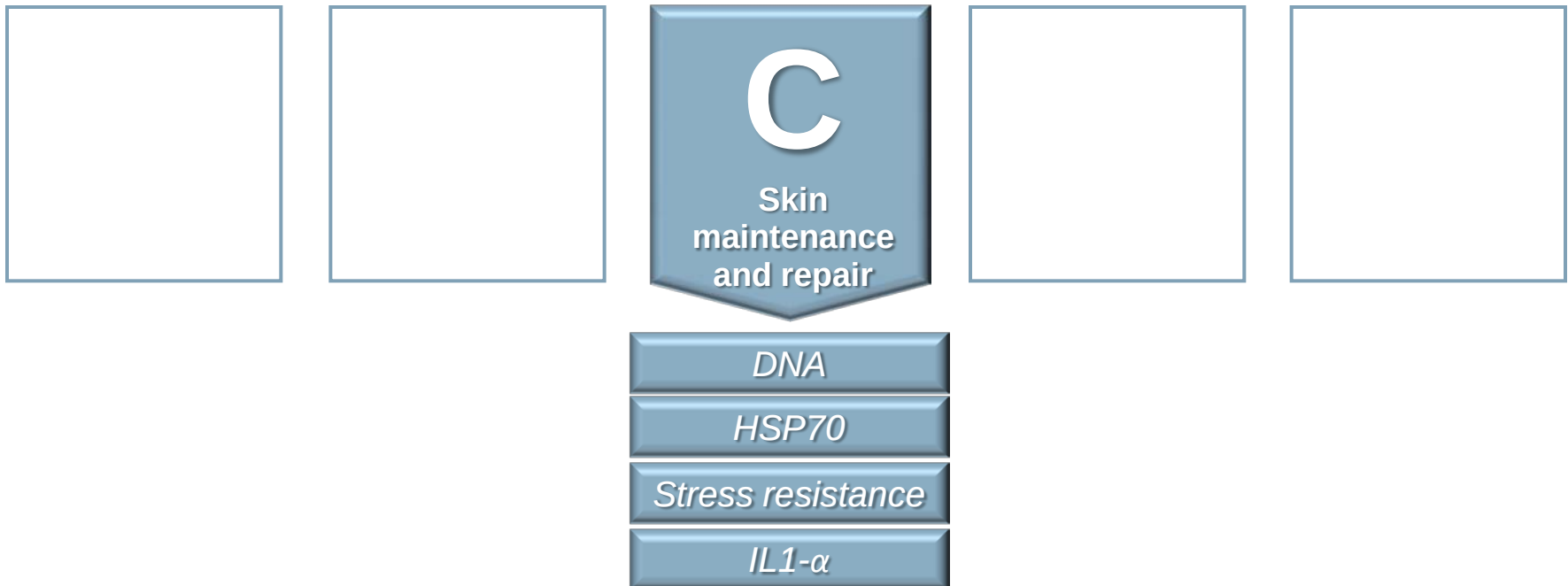
Application time: 24 hours

cAMP is a second messenger
which activates enzymes of
several metabolic pathways.



GP₄G SP helps boost the 2nd messenger cAMP in cutaneous cells (*in vitro*). cAMP is known to be involved in cellular energy metabolism.

GP₄G SP biofunctional



GP₄G SP biofunctional



Skin maintenance and repair: DNA

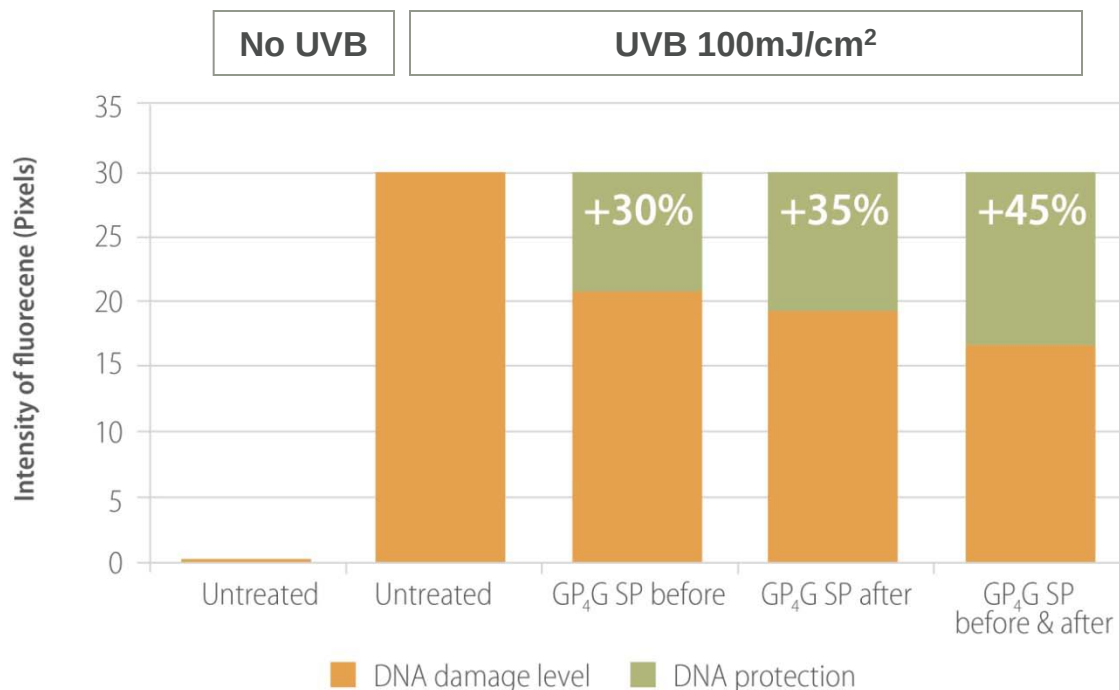
Cells: HaCaT cells

Use level: Biofunctional at 1%

Stress: UVB at 100mJ/cm²

Evaluation: Tunel assay method

Application time: Cells treated with or without biofunctional for 24 hours
-> UVB -> cells cultured with or without biofunctional for 24 hours.



GP₄G SP helps limit UV-induced DNA damage in cutaneous cells *in vitro*.

GP₄G SP biofunctional



Skin maintenance and repair: DNA

Cells: Human fibroblasts

Use level: Biofunctional at 3%

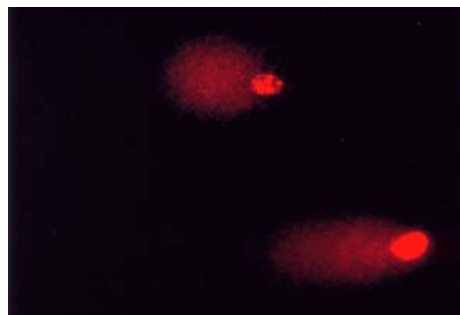
Application time: 48h

UVB dose: 30 mJ/cm²

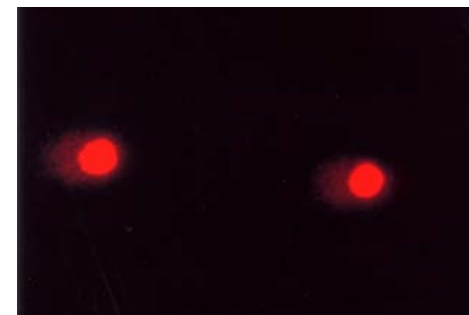
Evaluation: Comet assay

The comet assay is a very sensitive method to examine DNA damage and repair. DNA damage is evaluated by the size of the tail of the comet, in relation to the head (undamaged DNA).

Under low doses of UVB, when cells were treated with GP₄G SP, very little DNA damage is observed.

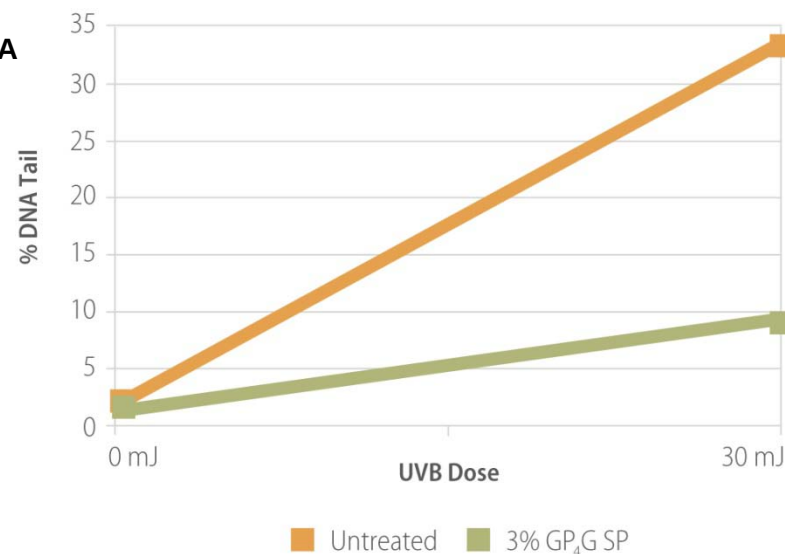


Irradiated cells



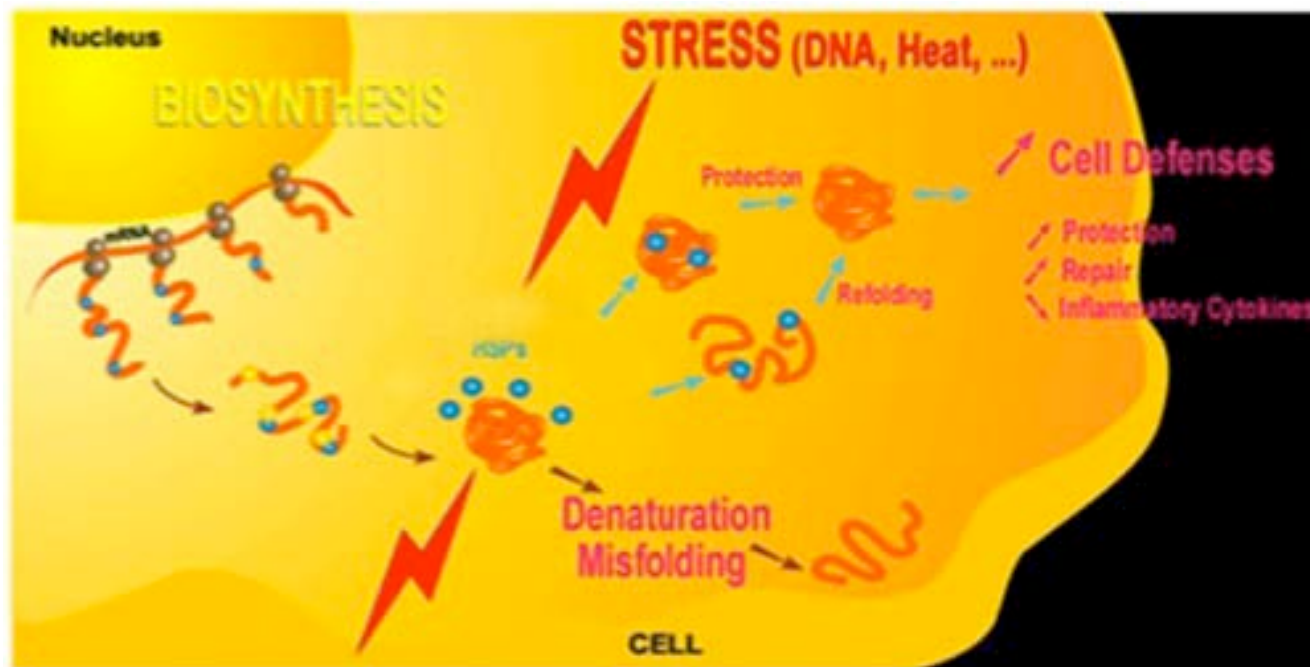
Irradiated cells treated with GP₄G SP

Percentage of DNA degradation



Heat Shock Proteins (HSPs)

Heat Shock Proteins (HSPs) or molecular chaperones, play an important role in protecting the cell from different types of stress.



GP₄G SP is shown to increase HSP70, a key protein for skin maintenance and repair, in various *in vitro* and *ex vivo* conditions.

GP₄G SP biofunctional

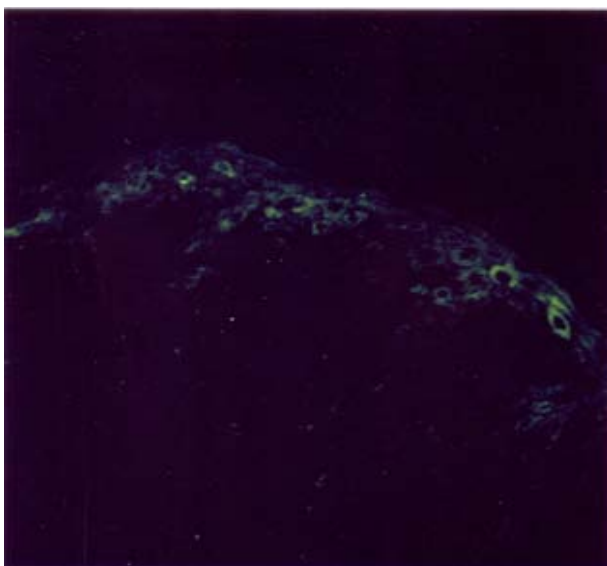


Skin maintenance and repair: HSP70

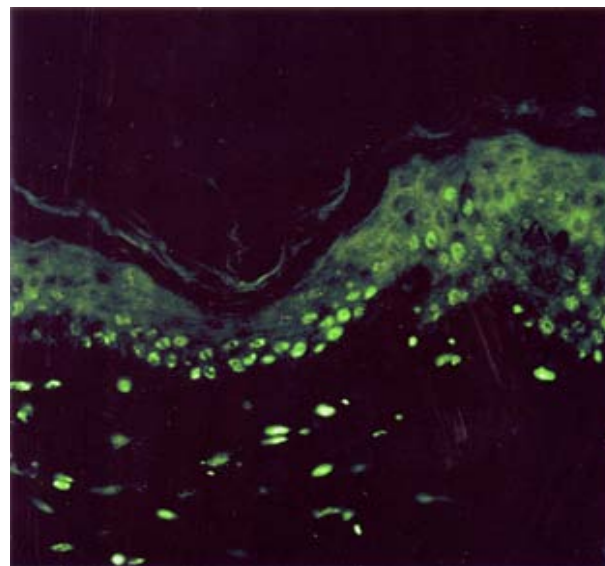
Culture: *Ex vivo* human skin

Use Level: Biofunctional at 3%

Evaluation: HSP70 Immunofluorescence staining (green) **Application Time:** 24 hours



Skin treated with Placebo



Skin treated with GP₄G SP

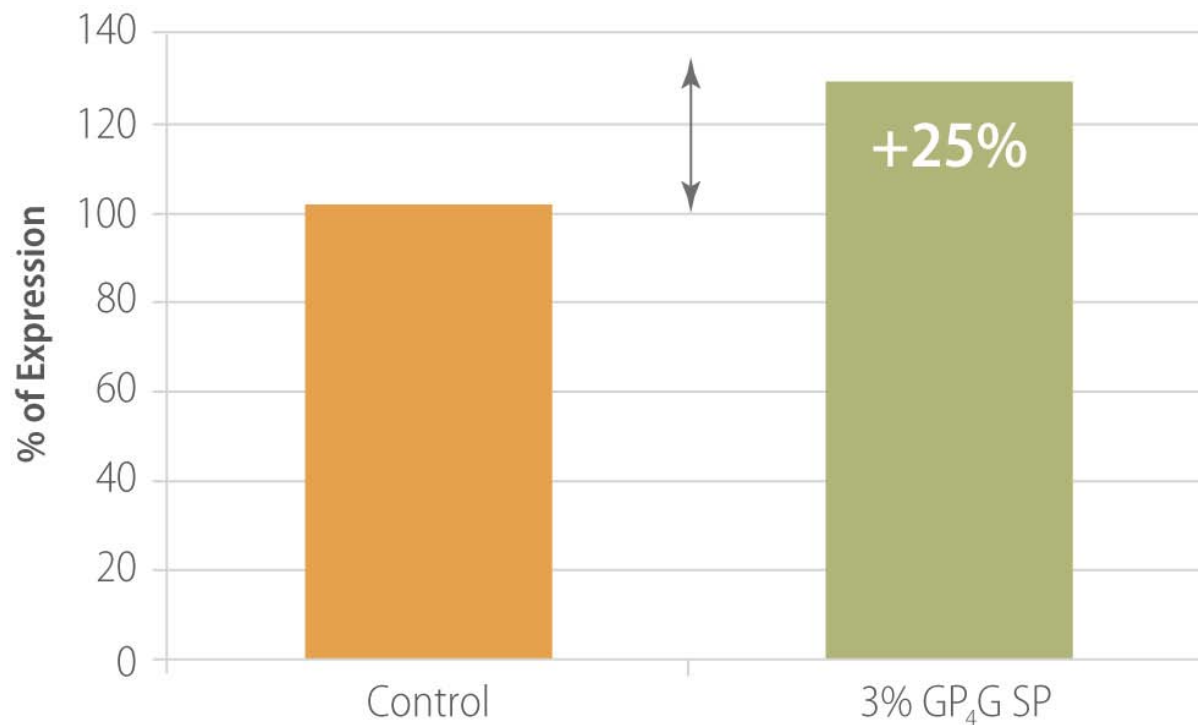
GP₄G SP **increases HSP70 expression** in human skin *ex vivo*, in stress-free experimental conditions.

GP₄G SP biofunctional



Skin maintenance and repair: HSP70

Cells: Human fibroblasts
Use level: Biofunctional at 3%
Application time: 24 hours
Evaluation: Immunoblotting



GP₄G SP also increases HSP70 expression in human fibroblasts, *in vitro*.

GP₄G SP biofunctional



Skin maintenance and repair: HSP70

Skin model: *Ex vivo* human skin

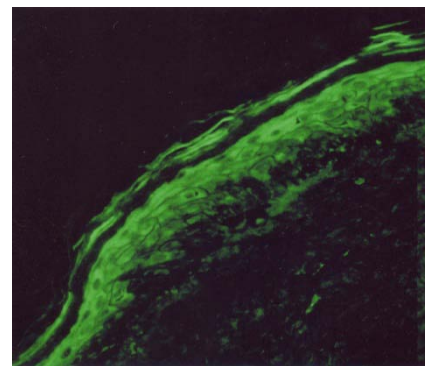
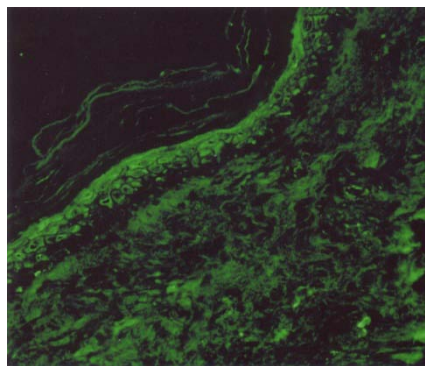
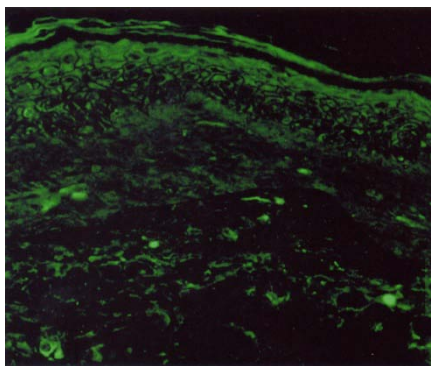
Use level: Biofunctional at 3%

UVB dose: 100 mJ/cm²

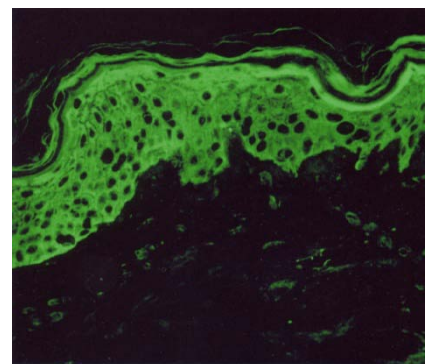
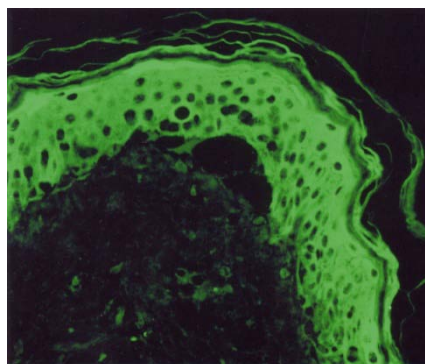
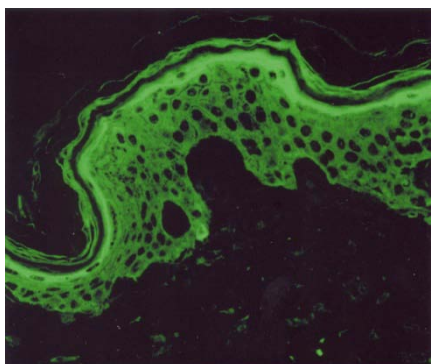
Evaluation: HSP70 Immunofluorescence staining (green)

Application time: 24 hours pretreatment → UVB stress → 24 hours treatment

Skin from
53 year old woman



Skin from
31 year old woman



Control, unirradiated skin

UVB

UVB + GP₄G SP

GP₄G SP helps limit age-related decrease in HSP70 in UVB stress conditions.

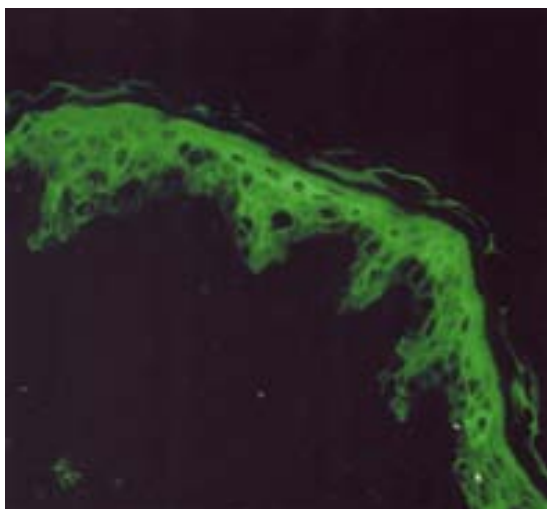
GP₄G SP biofunctional



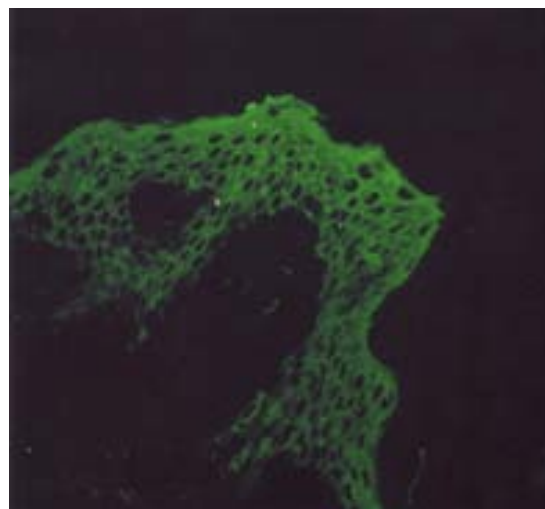
Skin maintenance and repair: HSP70

Skin model: *Ex vivo* human skin
ATRA dose: 10⁻² M

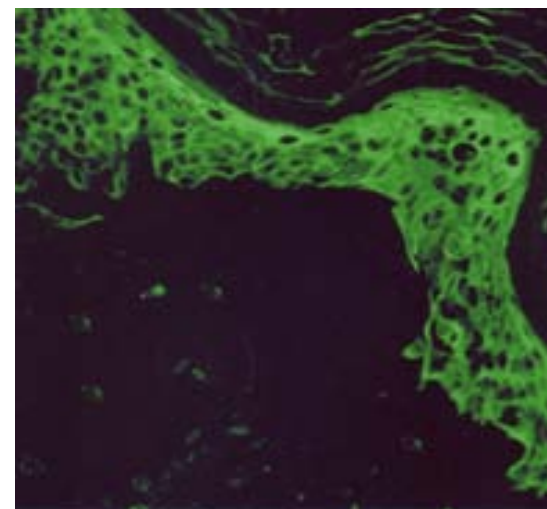
Use level: Biofunctional at 3%
Evaluation: Immunofluorescence



Control skin



After 5 days of ATRA
treatment



After 5 days of ATRA
& GP₄G SP at 3%

ATRA (All Trans Retinoic Acid) application on *ex vivo* skin decreases HSP70 content in skin cells. GP₄G SP helps skin restore HSP70 content in ATRA-treated cells *ex vivo*.

GP₄G SP biofunctional



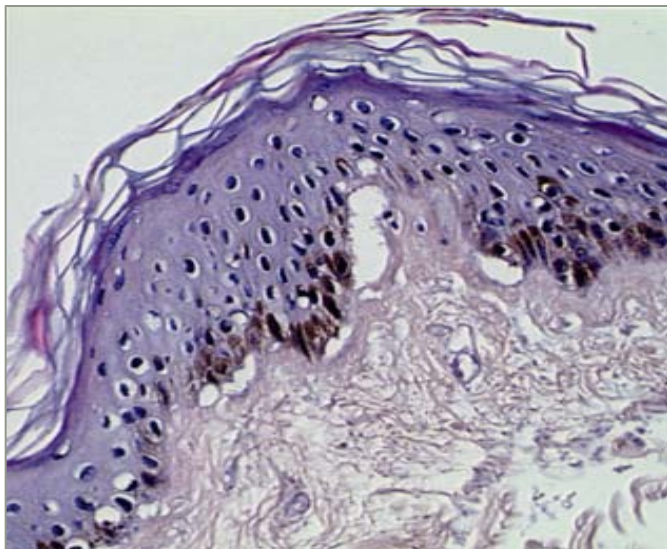
Skin maintenance and repair: stress resistance

Skin model: *Ex vivo* human skin

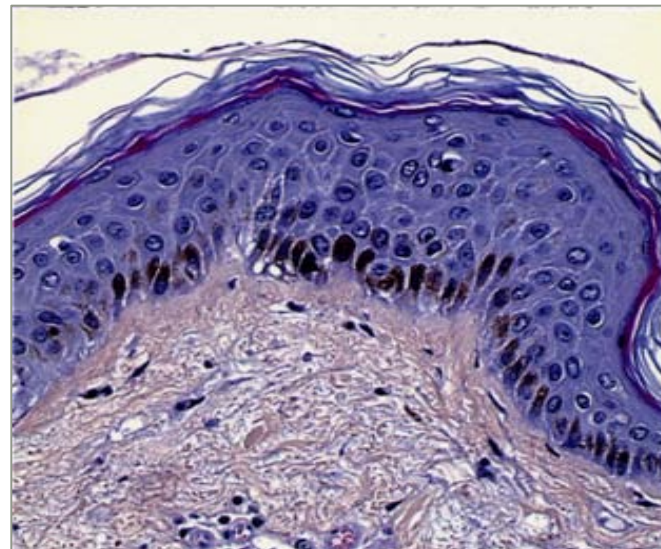
Heat stress: 47°C for 1 h, 37°C for 24 h

Use level: Biofunctional at 3%

Evaluation: H&E staining



Skin treated with placebo at 47°C



Skin treated with biofunctional at 47°C

The skin biopsy exposed to heat stress and treated with GP₄G SP shows almost no signs of stress, compared to the placebo-treated skin. GP₄G SP may help boost skin resistance to stress such as heat.

GP₄G SP biofunctional



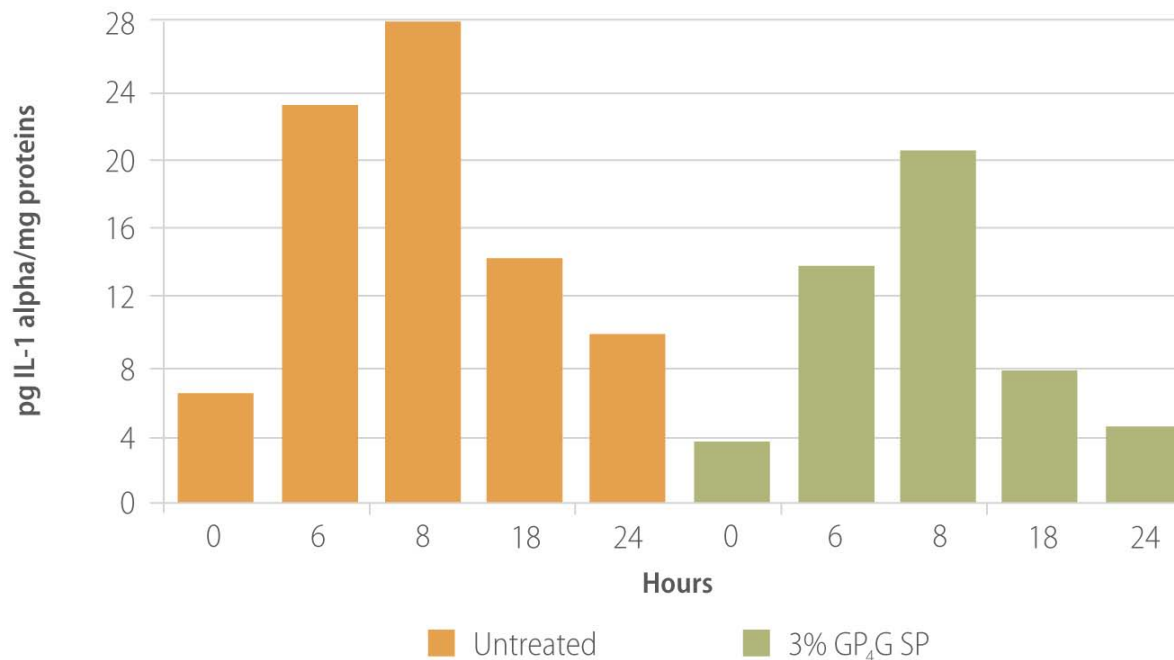
Skin maintenance and repair: IL-1 α

Cell culture: HaCaT cells

UVB dose: 30 mJ/cm²

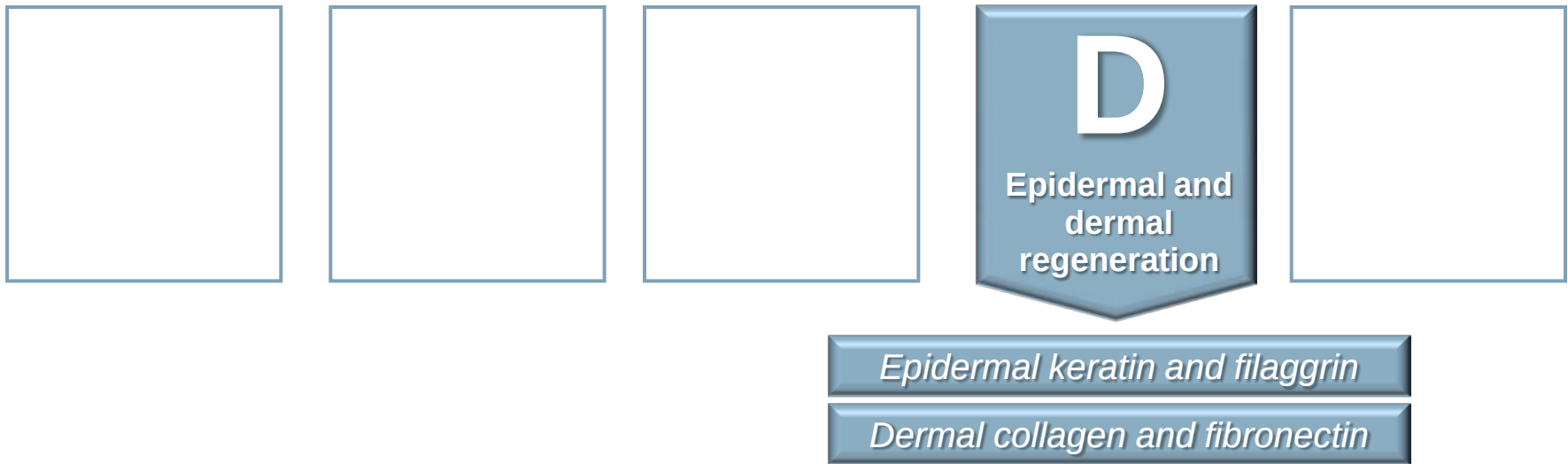
Use level: Biofunctional at 3%

IL-1 evaluation: Elisa assay



GP₄G SP helps limit UV-induced IL-1 α .

GP₄G SP biofunctional



GP₄G SP biofunctional



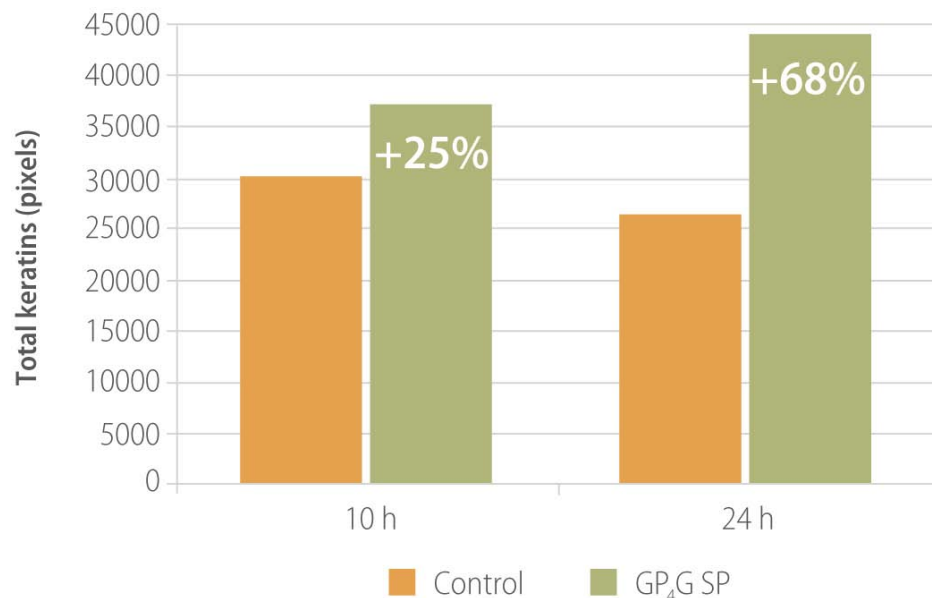
Epidermal regeneration: keratin, filaggrin

Cells: HaCat keratinocytes

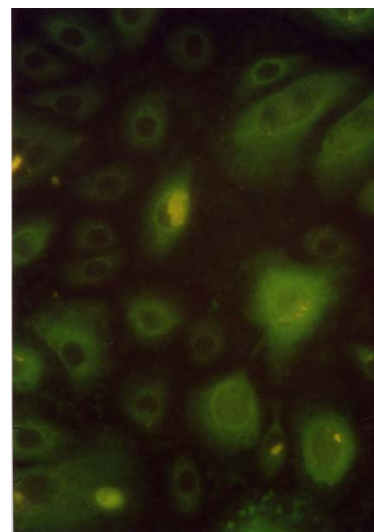
Use level: Biofunctional at 1%

Evaluation: Immunoblotting (keratin) and immunofluorescence (filaggrin, green staining)

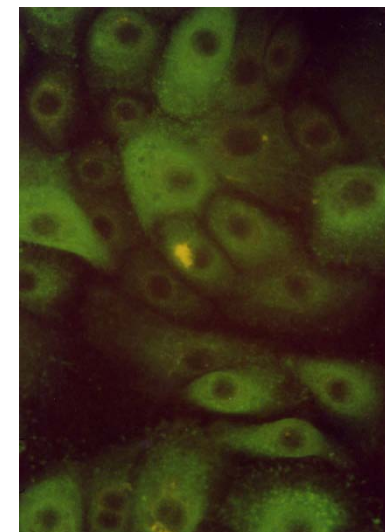
Keratin evaluation



Filaggrin evaluation



Control



GP₄G SP 1%

In vitro, GP₄G SP increases keratin and filaggrin expression.

GP₄G SP biofunctional

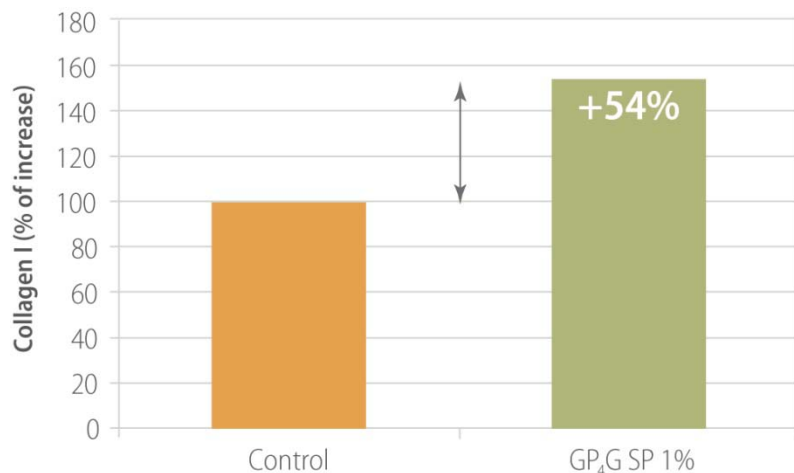


Dermal regeneration: collagen, fibronectin

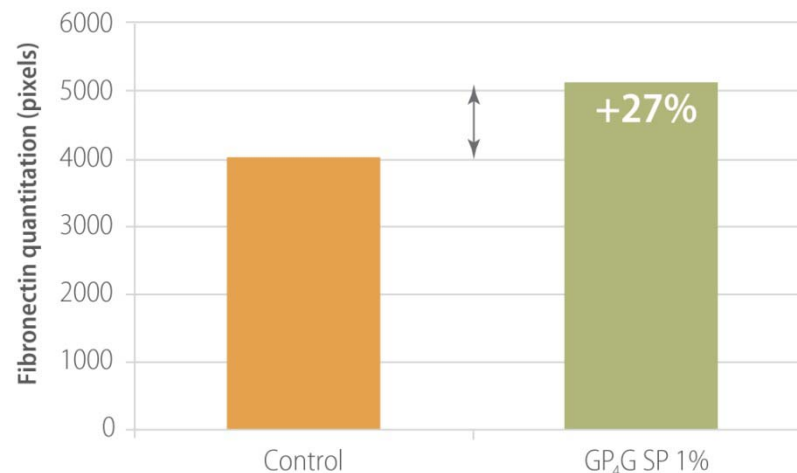
Cells: Human fibroblasts
Evaluation: Immunoblotting

Use level: Biofunctional at 1%
Application time: 48 hours

Collagen I evaluation

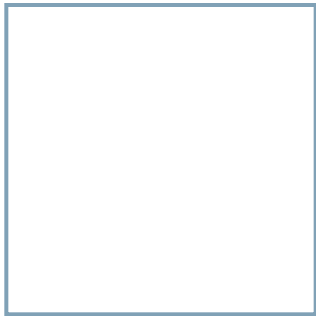
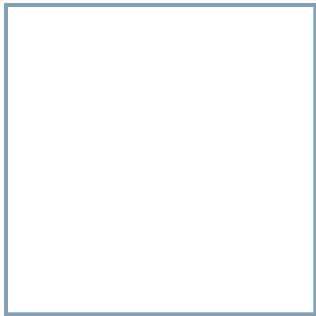


Fibronectin evaluation



GP₄G SP helps increase dermal collagen and fibronectin expression in fibroblasts (*in vitro*).

GP₄G SP biofunctional



GP₄G SP biofunctional



Cosmetic applications

- Revitalizing or energizing skin care products
- Day creams to help skin maintenance and repair
- Anti-photoaging cosmetic skin care
- Sun products to help skin limit UV-induced DNA damage and heat stress
- Skin protection and defense against environmental stress (HSP)
- In synergy with other biofunctionals
- Recommended use level: 0.5 - 5%

Disclaimer

The information contained in this brochure and the various products described are intended for use only by persons having technical skill and at their own discretion and risk after they have performed necessary technical investigations, tests and evaluations of the products and their uses. This material is for informational purposes only and describes the scientific support for the use of GP₄G SP biofunctional as an ingredient in cosmetic products intended to enhance appearance and other cosmetic benefits. While the information herein is believed to be reliable, we do not guarantee its accuracy and a purchaser must make its own determination of a product's suitability for purchaser's use, for the protection of the environment, and for the health and safety of its employees and the purchasers of its products. Neither Ashland nor its affiliates shall be responsible for the use of this information, or of any product, method, formulation, or apparatus described in this brochure. Nothing herein waives any of Ashland's or its affiliates' conditions of sale, and no statement, information and data is to be taken as a guarantee, an express warranty, or an implied warranty of merchantability or fitness for a particular purpose, or representation, express or implied, for which Ashland and its affiliates assume legal responsibility. We also make no warranty against infringement of any patents by reason of purchaser's use of any information, product, method or apparatus described in this brochure.

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