FIRM'ACT®

Targets adaptive defensive stress response through hormesis-based prevention

Ways of action

Up-regulates the gene expression of unique markers involved in the adaptive cellular stress response for maximizing protective effects

Strengthens the dermal structure for boosting the extracellular matrix maintenance

Clinical studies

Increases skin antioxidant capacity & protects against UV-induced damage

Delays facial skin sagging & increases skin firmness

For a more toned and better protected skin

Gelyma

An hormetic stressor capable to elicit efficient stress response for healthy and younger looking face.

The accumulation of damage occurring during ageing is linked to a failure of the homeodynamic, the dynamic version of homeostasis.

According to Professor Suresh I. S. Rattan (from Aarhus University, Denmark), the homeodynamic space is a kind of survival/buffering capacity which determine survival, quality and duration of lifespan. It can be thought of as our life-force.

More precisely it is the ability of living systems to respond to internal and external stress *via* an effective stress response and to counteract by neutralization and/or by adaptation any disturbances threatening their survival via damage control and management through a constant remodeling and adaptation during the lifespan.

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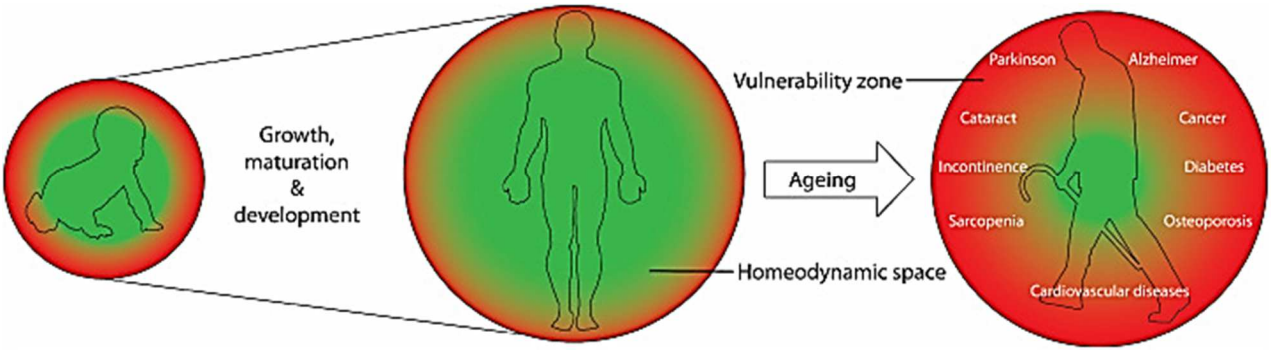


The progressive shrinkage of the homeodynamic space during ageing leads to an increased zone of vulnerability (red area on the Figure) and the onset of age-related diseases. It also works at the cellular level.

Skin ageing appears as a complex biological process influenced by a combination of intrinsic and extrinsic factors that acts on the homeodynamic space.

A failure of this space:

- induces changes in skin appearance (*e.g.* wrinkling, pigmentation irregularities, the appearance of neoplastic skin lesions)

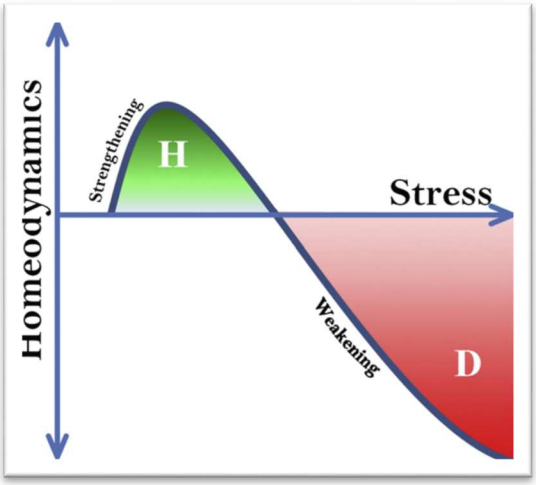
- leads to molecular damage and to the impairment in functional ability at all levels of organization in each key skin layer: epidermis, dermis and subcutaneous tissue..

Therefore it is important to able to modulate stress response *via* the outcome of a balance between damage and repair modulated by stress response and repair pathways linked especially to exposure to ultraviolet radiation and various environmental pollutants (*e.g*. volatile organic compounds, particulate matter, smokes, ozone, heavy metals…).

Several interpretations are being tried to understand such mechanisms in order to obtain stability through dynamic interactions of maintenance and repair.

A recent promising strategy for fighting skin ageing concerns the application of the concept of hormesis, well known in ageing research and based on the principle of stimulation of maintenance and repair pathways for counterbalancing stress, which better prepare skin cells against stronger insults

(cf. Rattan S.I. et al. 2013- Dose-response 11:99-108 , Rattan S. 2015- Cosmetics 2:11-20).



The homeodynamic ability of a biological system is affected by stress in a biphasic dose response manner, termed physiological hormesis.

Lower levels of stress result in the strengthening of homeodynamics in a hormetic zone (H).

Chronic and severe stress result in the weakening and disruption (D) of the homeodynamics leading to functional impairments, diseases and eventual death.

From Demirovic & Rattan 2013- Experimental Gerontology 48 : 94-98.

Depending on the severity and duration of stress encountered, cells react to various stresses through a number of self-maintenance and repair pathways to counteract damaging effects in cells. These pathways may include *e.g*. induction of Nrf2-based SR pathways, induction of NFkB pathways, DNA damage response, induction of HSP 70 or autophagic and sirtuin-dependent SR pathways

(cf. in Rattan S.I. & D. Demirovic 2010 a- in: Everitt, AV, Rattan, SIS, Le Couteur, DG and de Cabo, R. (eds.), Calorie Restriction, Aging and Longevity, pp. 233-245, Springer, The Netherlands ; 2010 b- Dose Response 8: 58-63).

In fact these stress response pathways are quite specific, not all responding to every stressor. The specificity of the response is mostly determined by the nature of the damage induced by the stressor and the variety of downstream effectors involved.

Applying the idea of beneficial effects initiated by the hormesis concept, GELYMA proposes FIRM'ACT® for fighting both intrinsic and extrinsic skin ageing through several adaptive abilities against various kinds of stress, especially oxidative stress and metals-induced stress.

FIRM'ACT® combines two extracts prepared from the brown seaweeds *Himanthalia elongata* and *Fucus vesiculosus* with a specific extract of *Saccharomyces cerevisiae* supplemented with selenium, each one complementing one another specially in anti-oxidant properties.

FIRM'ACT® offers a natural innovative way to the resistant skin cell stress response by coordinating defensive cellular stress response *via* the over expression of unique markers (such as metallothioneins MT-1 and MT-2) for maximizing cellular adaptation and survival.

FIRM'ACT® also is able to strengthen the dermal structure by stimulating fibroblast proliferation and increasing collagen and hyaluronic acid synthesis for boosting the extracellular matrix maintenance.

Visible cosmetic benefits for improving the aesthetic appearance of the facial skin have been confirmed by two sets of clinical studies (*versus* placebo) and consumer use assessment.

Mechanisms of action

The mechanisms of action of FIRM'ACT® has been demonstrated by using the transcriptomic analysis (collaboration Strati CELL-Belgium) completed by other *in vitro* and Elisa testing (collaboration SEPhRA PHARMA-France) and clinical studies (collaboration FARCODERM-Italy) in order to provide valuable data.

FIRM'ACT ® modulates gene expression of unique markers involved in the adaptive cellular stress response

Method: *Transcriptomic analysis on normal human fibroblasts cultured in depleted medium with 5% FIRM'ACT® (no cytotoxic dose) added for 24h (n=3). Analysis by qRT-PCR on TaqMan cards (96 genes).*

➤ The effect of FIRM'ACT® on MT-1 & MT-2 gene expression

Metallothioneins, especially MT-1 and MT-2, are known for their unique influence on both the generation of free radicals and the heavy metals detoxification process as well as their beneficial role in photoprotection.

Moreover it is proved that MT expression decreases with old ageing and so the modulation of MT may be beneficial for restoring the immune function at old age.

➤ By over-expressing MT-1 & MT-2 genes, FIRM'ACT® enhances cellular defense against different kinds of aggressions (heavy metals, reactive oxygen species, UV).Therefore FIRM'ACT® helps prevent both extrinsic and intrinsic skin ageing.

Gene abbreviation	Gene name	Expression change*	P value (t-test)
MT1G	Metallothionein -1G	79.82	0.0008
MT1H	Metallothionein -1H	24.88	0..0035
MT2A	Metallothionein 2A	1..29	0.0494

*relative expression compared to untreated control

➤ The effect of FIRM'ACT® on HSPA1A gene expression

The HSPA1A gene encodes a 70 KDa heat shock protein 1 known for its contribution to various biological process e.g. signal transduction, apoptosis, protein homeostasis, antioxidant stress, cell growth and differentiation.

➤ FIRM'ACT® comforts the antioxidant response & maintains protein homeostasis allowing cells to resist to denaturation of cellular proteins.

Gene abbreviation	Gene name	Expression change*	P value (t-test)
HSPA1A	Heat shock 70 kDa protein 1A	1.29	0.0107

*relative expression compared to untreated control

➤ The effect of FIRM'ACT® on NQO1 gene expression

The NQO1 gene encodes a flavoenzyme playing a pivotal role in monitoring cellular redox state. It is also considered as a potent detoxification enzyme with a broader scavenger function protecting cellular membranes against oxidative stress.

➤ FIRM'ACT® increases the antioxidant defence, maximizes skin detoxification systems and regulates the cellular redox state.

Gene abbreviation	Gene name	Expression change*	P value (t-test)
NQO1	NAD(H) dehydrogenase quinone 1	1.95	0.0016

*relative expression compared to untreated control

➤ FIRM'ACT® is able to activate anti-stress gene regulatory network for stimulating a cascade of "homeostatic pathways" *i.e.* adaptive response which help reinforce cell detoxification and protection from stress harmful environments. That maximizes cellular adaptation for a better skin preparation against aggressors damage.

FIRM'ACT® strengthens the dermal structure

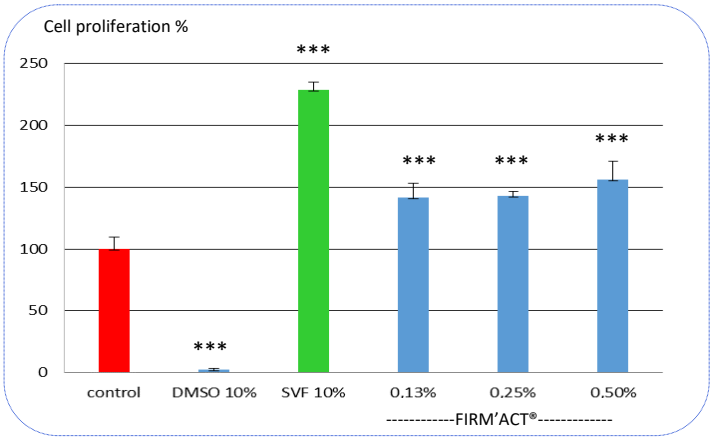
The structural integrity and function of the dermis depend on its extracellular matrix (ECM) composed by different molecules secreted in particular by fibroblasts. During skin ageing, changes concern fibroblasts as well as the major molecules of the matrix.

Methods:
In vitro studies on normal human fibroblasts cultured in depleted medium (stress conditions).

➤ FIRM'ACT® is able to act on both fibroblasts and some ECM components, especially Type I collagen and hyaluronic acid.

➤ The effect of FIRM'ACT® on fibroblast proliferation (*Demonstration by different tests*).

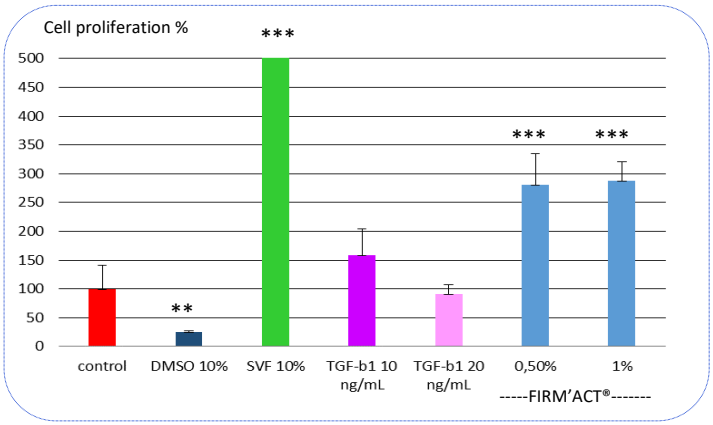
XTT testing



XTT testing is commonly used for quantitating and viabilities studies of cells.

The cell proliferation increases by
+ 43 % with 0.25 % FIRM'ACT®
+ 56 % with 0.5 % FIRM'ACT®
versus control.

BrdU testing



The BrdU testing is used for cell proliferation study and also for measuring DNA synthesis.

The cell proliferation increases by
+ 180 % with 0.5 % FIRM'ACT®
+ 188 % with 1 % FIRM'ACT®
versus control.

Transcriptomic analysis

Gene abbreviation	Gene name	Expression change*	P value (t-test)
MKI67	Antigen Ki-67	1.64	0.0175

*relative expression compared to untreated control

The ki-67 is a cellular marker for proliferation. It is associated with ribosomal RNA transcription.

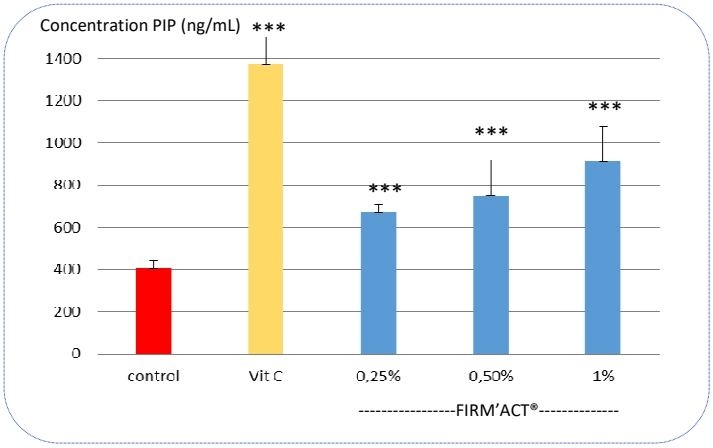
The over-expression of MKI67 confirms the ability of FIRM'ACT® to promote fibroblast proliferation.

➤ FIRM'ACT® highly significantly stimulates the capacity of fibroblasts to proliferate and therefore improves skin vitality. (good correlation between the different testing).

➤ The effect of FIRM'ACT® on the molecules of the extracellular matrix

Stimulation of the Type I collagen synthesis

Collagen is a key structural component of the skin matrix giving the tissue their mechanical strength and thus participates in maintaining their firmness. During skin ageing, the amount of Type I collagen decreases overall, thereby leading to reduction of skin strength and elasticity which in turns may lead to wrinkles and sagging.



The synthesis of Type I pro collagen increases by

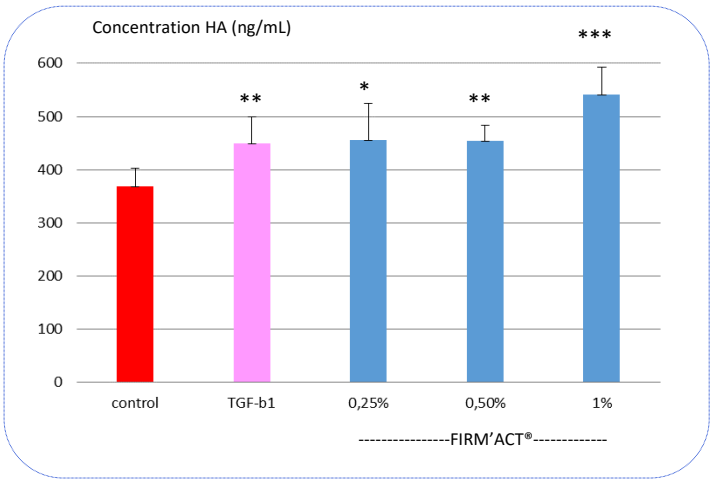
+ 65 % with 0.25 % FIRM'ACT®
+ 84 % 0.5 % FIRM'ACT®
+ 125 % 1 % FIRM'ACT®

versus control.

➤ By highly significantly stimulating the Type I collagen synthesis, FIRM'ACT® is able to increase skin resistance.

Stimulation of the hyaluronic acid synthesis

Hyaluronic acid (HA) plays a pivotal role for the maintenance of highly hydrated extracellular matrix. In the dermis it functions as a sieve, excluding certain molecules, enhancing the extracellular domain of cell surfaces and stabilizing skin structures by electrostatic interactions.



The synthesis of hyaluronic acid increases by

+ 24 % with 0.25 % FIRM'ACT®
+ 23 % 0.5 %
+ 47 % 1 %

versus control.

➤ By significantly stimulating the hyaluronic acid synthesis, FIRM'ACT® help regulate skin moisture while improving skin smoothness.

Transcriptomic analysis

MMP1 is involved in the breakdown of the extracellular matrix, specially of collagens.

Gene abbreviation	Gene name	Expression change*	P value (t-test)
MMP1	Matrix metalloproteinase 1 (interstitial collagenase)	0.57	0.0053

*relative expression compared to untreated control

➤ By down-expressing the MMP-1 gene expression, FIRM'ACT® prevents collagen degradation.

➤ FIRM'ACT® stimulates the synthesis of two major matrix molecules, thus strengthens the dermal structure and boosts matrix maintenance.

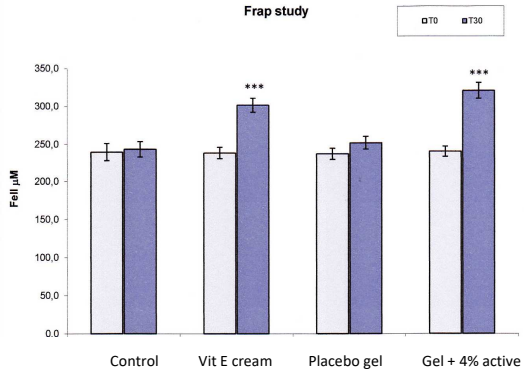
Clinical studies

FIRM'ACT® increases skin antioxidant capacity

Method: *Clinical studies by tape striping on 20 women Caucasian volunteers from 30 – 60 years old with various phototypes (II:7 subjects - III : 9 subjects - IV: 4 subjects). Treatments with products applied twice daily for 28 days on thigh. UV radiation (5 J/cm²) at D0 and D 30. FRAP assay measuring the total reductive power and indirect antioxidant capacity (stripping at T30 + 4h and T30 + 24 h).*

Results reported as Fe2+ µM ± SEM.

	Δ D O / D 30
Control (untreated area)	3.5 %
Vitamin E (2%) cream	26.9 %
Placebo	6.8 %
Gel 4% FIRM'ACT®	33.5 %



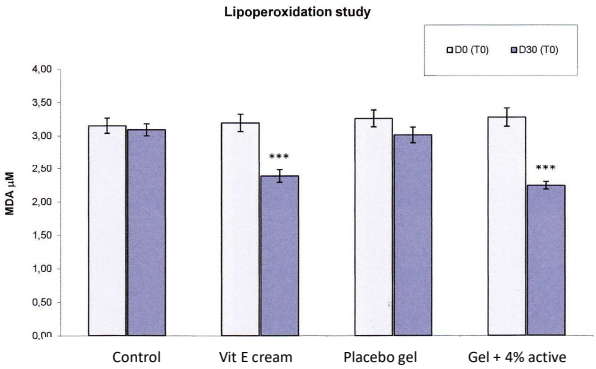
➤ FIRM'ACT® induces a highly statistically significant improvement of skin antioxidant capacity at T30 *versus* T0.

FIRM'ACT® protects against UV-induced damage (skin lipid peroxidation)

Method: *Clinical studies by tape striping on 20 women Caucasian volunteers from 30 – 60 years old with various phototypes (II:7 subjects - III : 9 subjects - IV: 4 subjects). Treatments with products applied twice daily for 28 days on thigh. UV radiation (5 J/cm²) at D0 and D 30. MDA assay evaluating the lipo-peroxides levels, so skin protection against lipo-peroxidation (stripping at T0 - T30 + 4h and T30 + 24 h).*

Results reported as MDA µM ± SEM.

	Δ D 0 / D 30
Control (untreated area)	0.3 %
Vitamin E (2%) cream	-22.9 %
Placebo	-5.7 %
Gel 4% FIRM'ACT®	-29.4 %



➤ FIRM'ACT® induces a statistically significant reduction of basal lipoperoxidation damage at D 30 versus D0.

➤ After a period of use of 30 days (twice daily application), FIRM'ACT® in a basic gel at 4% induces:

- positive modulation (statistically significant vs D0) of the skin antioxidant capability (FRAP assay)

  ➤ FIRM'ACT® is able to increase the total antioxidant capacity of the skin in a statistically significant way compared to the untreated, placebo treated and vitamin E treated skin areas,

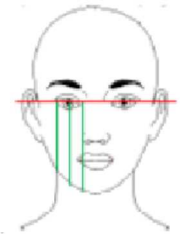
- reduction of both basal and UV-induced LPOs levels, demonstrating a protection against skin lipid peroxidation (MDA assay).

  ➤ FIRM'ACT® is able to decrease the lipoperoxide levels in a statistically significant way compared to the untreated and placebo treated skin areas.

➤ These results confirm the effective protective ability of FIRM'ACT® for enhancing cellular defense demonstrated by transcriptomic analysis.

FIRM'ACT® decreases skin sagging

Method: *Clinical studies performed on two groups of 20 volunteers, one testing placebo, the other a gel with 4% active.*



The lifting effect is assessed by means of a morphometric image analysis. The photographic images are taken using a digital camera.

Lifting effect is evaluated by measuring the distance (mean of the green lines) in cm between the line (red) passing between eyes and the lower side of the face. A reduction of this parameter is a sign of a lifting effect.

The table here after reports the mean of variations of sagging for volunteers between D 0 and D 28 days application of products.

	Δ D O / D 28
Placebo	0.04 %
Gel 4% FIRM'ACT®	-1.09 %

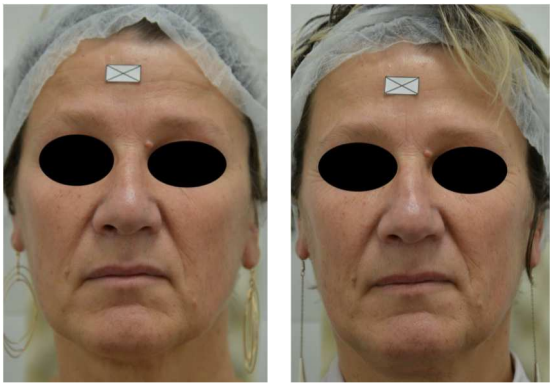
The variation (+ 0.04 %) relative to placebo gel is non statistically significant compared to baseline.
The variation (- 1.09 %) relative to the gel with 4 % active is statistically significant compared to baseline.

Volunteer 1 – 63 years

D O D 28

Δ D 0/D 28 – 4.05 %

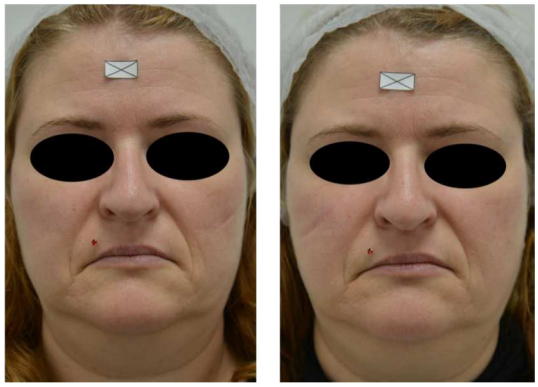
Volunteer 2 – 56 years



D O D 28

Δ D 0/D 28 – 3.56 %

Volunteer 3 – 47 years



D O D 28

Δ D 0/D 28 – 3.31 %

➤ FIRM'ACT® in a gel at 4% induces a significant reduction of skin sagging compared to placebo gel, improves visibly the face contour and offers a lifting effect.

FIRM'ACT® reinforces skin firmness and elasticity

Method: *Clinical studies performed on two groups of 20 volunteers, one testing placebo, the other a gel with 4% active.*

The measurement of the skin elasticity is based on skin suction/elongation principle after a negative and constant pressure stimulation and on its subsequent release when the stimulus finishes.

In this study the skin elasticity was calculated as the ratio between the residual deformation and the maximum elongation of the skin (R0) or Ua/Uf. This ration is known in literature as R2 and indicates the ability of the skin to return to its original state of recovery after a stressing event.

RO : skin extensibility

If R0 decreases the skin is less extensible, so more stretched.

	Δ D O / D 28
Placebo	- 6.1 %
Gel 4% FIRM'ACT®	-13.7 %

➤ FIRM'ACT® reduces the extensibility of the skin, so presents a tightening effect on the skin.

R2 : skin gross-elasticity

If R2 increases, skin elasticity increases.

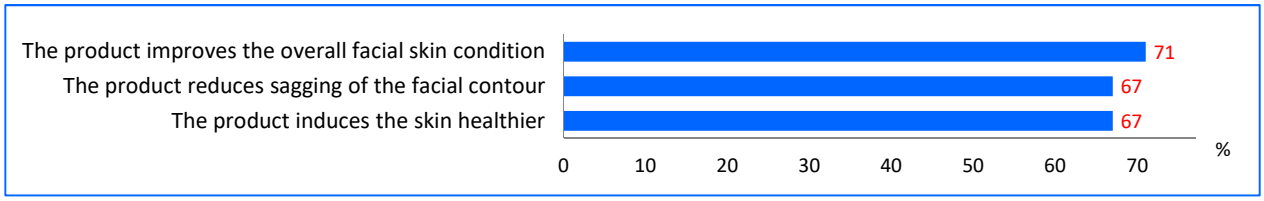
	Δ D O / D 28
Placebo	- 5.2 %
Gel 4% FIRM'ACT®	+ 5.9 %

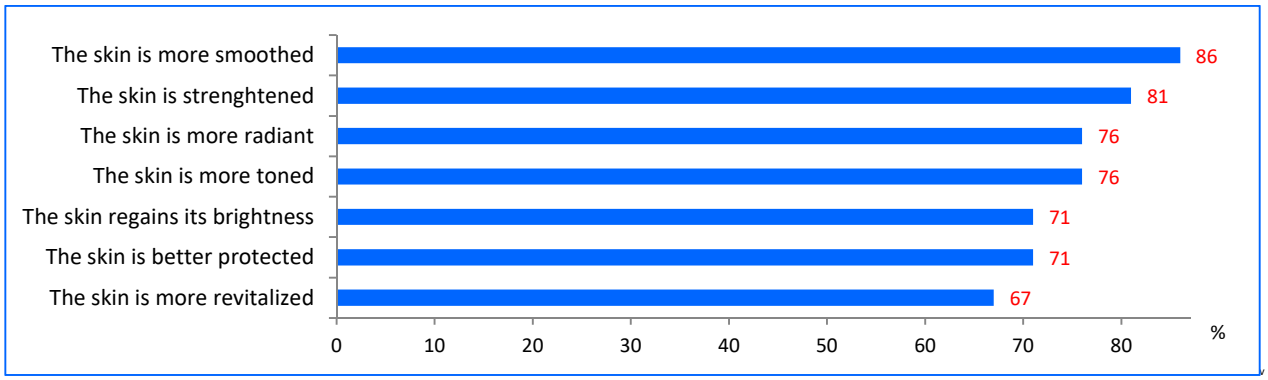
➤ FIRM'ACT® improves the elasticity of the skin

➤ By increasing skin extensibility and elasticity, FIRM'ACT® offers the ability of inducing skin tightness which prevents skin damage and delays the ageing process.

FIRM'ACT ® - subjective evaluation

The volunteers confirmed these results by their own assessment of the product's effectiveness after 28 days application.





Cosmetic benefits

FIRM'ACT® is a synergistic combination of two aqueous extracts prepared from the brown seaweeds

Himanthalia elongata
Fucus vesiculosus

with a specific extract of *Saccharomyces cerevisiae* supplemented with selenium, each one complementing one another in anti-oxidant properties.

FIRM'ACT® is based on the hormesis concept related to the principle of stimulation of maintenance and repair pathways for counterbalancing stress in order to ameliorate healthy skin state and better prepare skin cells from harmful agents.

It is well known that stress-induced damages induce deterioration of skin quality and play major role in accelerating skin ageing.

Consequently the hormesis concept is becoming a recent promising strategy for fighting skin ageing.

FIRM'ACT® offers a double preventive approach to skin anti-ageing. Its effectiveness relies on two major aspects:

- Protection against environmental stressors *via* unique adaptive abilities against various kinds of stress, especially oxidative stress and metals-induced stress.
  - FIRM'ACT® activates anti-stress gene regulatory network by coordinating defensive cellular stress response and stimulating a cascade of "homeostatic pathways" related to oxidative stress (HSPA1A - NQO1) and cell detoxification (metallothioneins MT-1 - MT-2) from harmful environments for maximizing cellular adaptation and survival.
- Improvement of the dermis maintenance.
  - FIRM'ACT® increases the cellular dynamism (stimulation of fibroblast proliferation) and comforts the molecule network (stimulation of Type I collagen and hyaluronic acid synthesis) that boosts the matrix architecture.

These cosmetic benefits have been confirmed by two sets of clinical studies and consumer use validation.

The first set of clinical studies concerns substantial skin protective effects of FIRM'ACT® related to its skin antioxidant capacity (FRAP assay) and its effective decrease of skin lipo-peroxidation (MDA assay) after twice daily application of a gel with 4% active for 28 days (tape stripping method on 20 women Caucasian volunteers versus placebo gel) .

The second set of clinical studies regards the lifting effect and firmness efficacy of FIRM'ACT® on 21 subjects for the evaluation of a gel with 4% active and 21 subjects for the evaluation of the placebo gel, after twice daily application for 28 days. FIRM'ACT® is able to redesign facial contours by remodeling sagging skin areas and reinforcing skin firmness and elasticity.

As results the overall facial skin condition is improved and contours lifted.

Cosmetic applications

- firming treatment , anti-sagging treatment – remodeling face contour treatment
- anti-ageing skin care

CHINA LISTED 2015

Recommended use level: 3% -5%.

Indicative formulation - *Cream facial care*

Phase	Ingredients	quantity en g	INCI names
A	Eau	55	Aqua (Water)
	Glycérine	3	Glycerin
	Rhodicare T	0,7	Xanthan Gum
	Sorbate de Potassium	0,3	Potassium Sorbate
	Benzoate de Sodium	0,3	Sodium Benzoate
B	Emulgade PL68/50	5,5	Cetearyl Glucoside (and) Cetearyl Alcohol
	Cithrol GMS 40	3	Glyceryl Stearate
	Huile de Macadamia	5	*MacadamiaTernifolia* Seed Oil
	Myritol 318	13	Caprylic/Capric Triglyceride
C	Vitamine E acétate	0,2	Tocopheryl Acetate
	Geogard 221	0,8	Dehydroacetic Acid (and) Benzyl Alcohol
	Parfum SHG60075	0,3	Perfume
	Extract FIRM'ACT®	4	
	Acide citrique à 50%	0,3 Qs pH 5,4 - 5,8	Citric Acid
	Eau	Qs 100	

This formula is presented in good faith, and we believe it is correct, but no warranty as to accuracy of results, or fitness for a particular use is given, nor is freedom from patent infringement to be inferred.

It is offered solely for your consideration, investigation and verification.

We are unable to guarantee the stability of this formula in view to limited stability studies.

Specifications

Aspect	limpid liquid orange-amber coloured with typical odour.
Odor	characteristic
pH	6.0 ± 1.0
Soluble	in aqueous solutions.
Insoluble	in oils.

Regulatory data

INCI names	CAS n°	EINECS n°	China compliant (list 2015)	
water	7732-18-5	231-791-2	06260	水
Himanthalia elongata extract	223751-70-0	-	06011	伸长海条藻（HIMANTHALIA ELONGATA）提取物
Fucus vesiculosus extract	84696-13-9	283-633-7	04728	墨角藻（FUCUS VESICULOSUS）提取物
Saccharomyces cerevisiae extract	84604-16-0	283-294-5	05064	啤酒酵母菌（SACCHAROMYCES CEREVISIAE）提取物
Preservative	as required	-		

Why is FIRM'ACT® so unique ?

Origin/ description

FIRM'ACT® combines a specific extract of *Saccharomyces cerevisiae* with two brown seaweeds extracts prepared from *Himanthalia elongata* and *Fucus vesiculosus*.

The extract of *Saccharomyces cerevisiae* used in FIRM'ACT® is a selenium-yeast extract (Se-Ye) produced by fermenting *Saccharomyces cerevisiae* in a selenium-rich media and chosen due to its interesting chemical composition linked to exceptional anti-oxidant properties.
A such selenium yeast extract is known to offer exceptional value for animal and human nutrition but also for crop nutrient and cosmetics. Since 2000, selenium yeast has received numerous approval for diets from US Food and Drugs Administration (FDA), European Food Safety Authority (EFSA).
In fact in this selenium-yeast extract, selenium is organically bound into proteins mostly *via* two selenium-containing amino acids: selenocystein and selenomethionin. Main selenoproteins in cells are enzymes showing antioxidant properties like glutathione peroxidase and thioreductase.

Morphology of *Himanthalia elongata in situ*
Photo Gelyma

Himanthalia elongata is a brown seaweed belonging to the kingdom *Chromista*. Its thallus is sharply differentiated into two morphological stages like vegetative (small button-like thallus) and reproductive (longs fronds).

Himanthalia elongata is seen as European endemic species. It can be found along the coasts of Northern Atlantic from Norway to Northern Spain and Portugal. It does not occur in the Pacific.

It is known to be rich in sodium, potassium and magnesium and capable to scavenge free radicals. Proved antioxidant properties would be linked to the presence of fucoxanthin or the presence of phlorotannin. This alga known as "sea spaghetti" is commonly eaten in France and Ireland.

Himanthalia elongata here present provides minerals and anti-oxidant properties.

Morphology of Fucus *vesiculosus in situ*
Photo Gelyma

The brown seaweed *Fucus vesiculosus* belongs to the kingdom *Chromista* too.This algal species is easy to recognize by its bladders along the fronds. These bladders (air vesicles) help the alga to stand straight up in the water.

Fucus vesiculosus is common in the North Atlantic to the Canary Islands, Morocco and Madeira. It is also found on the Atlantic coasts of North America from Ellesmere Island, Hudson Bay to North Carolina.

It is known to offer beneficial properties for numerous applications.

As medical applications, it was already known by the Romans. From the 16th century on, it served in China to treat goitre caused by iodine deficit. It is included in the *Pharmacopoea Gallica* since 1818 (French Codex) and in the positive list for human consumption accepted by the French National Council for Health since 1988. In addition to anticellulite activity this alga is known to inhibit thermally induced collagen glycation and to offer anti-collagenase and anti-elastase properties. It is proved that it increases skin thickness and elastic properties suggesting efficient anti-ageing activities.

The extract of *Fucus vesiculosus* present in FIRM'ACT® brings polyphenols and fucoidan, so it supports anti-ageing effects and comforts the anti-oxidant properties of *Himanthalia elongata* and *Saccharomyces cerevisiae extract.*

The 3 components present in FIRM'ACT® acts in synergy to maximize skin protection and combat skin ageing and the oxidative stress and so contribute to healthy looking skin.

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