

**CODIF**  
R&N

# LAKESIS

The Precious Tear  
that reactivates KLOTHO







# LAKESIS

## AWAKES THE YOUTH PROTEINS OF THE SKIN

On the island of Chios, Greece, ancient trees produce a resin which drops from the trunk into the shape of tears. Tears of Chios have endless cosmetic and medicinal properties. Once crystallized, they are used for the production of our Anti-Ageing ingredient: LAKESIS.

LAKESIS increases the synthesis of two youth proteins: KLOTHO and FOXO. 54-year-old fibroblasts recover characteristics of 37-year-old fibroblasts. Cellular detoxification processes are reactivated, their global cellular activity is increased, and we observe a re-densification of the dermis in fibrillin and collagen fibers.

Redensified, the skin looks younger, thicker and facial contours appear to be redrawn.

## ENTER THE LEGEND...

LAKESIS refers to the Greek legend of the Three Fates: Clotho, Lachésis and Atropos. According to legend, Clotho spins the thread of life onto a spindle. Lachesis (“the allotter”) measures its length and Atropos (“the inexorable”) cuts this thread with her scissors, signifying the end of life.

The goddess Clotho gave her name to the KLOTHO protein identified in 1997 and able to slow-down aging. The Precious Oil of Crystal Tears increases the synthesis of Klotho protein in the fibroblasts. In reference to the legend, we have called this oil Lakesis, from the name of the second goddess who measures lifespan.

## THE LEGEND OF CHIOS TEARS...

The trees where these tears drop off are called *Pistacia lentiscus*.

*Pistacia lentiscus* is native throughout the Mediterranean region but the only place where these trees elaborate their resin tears is in Chios Island (Greece). A medieval legend explains that the trees started crying resin tears, as an expression of lament and sadness when Agios Isidoros, an Egyptian living in Chios and who served the Roman army during the rule of the Roman emperor Decius, was severely tortured by the Romans on the island for worshipping at a Christian church.

The cultivation of *Pistacia lentiscus* on Chios Island started in the ancient times. This resin is used for the elaboration of different products, especially a very unique chewing gum, as well as quality spices, alcoholic beverages, sweets and cosmetic products. The medicinal benefits of Chios tears are almost endless.

# YOUTH PROTEINS

## WHAT ARE THEY?

## WHAT ROLE DO THEY PLAY IN CELLULAR LONGEVITY?

### THE YOUTH PROTEIN FOXO

Its role in cellular protection and longevity has been well described and targeted to combat aging. Located directly inside the nucleus, the “nuclear” form of FOXO activates the transcription of genes involved in cellular detoxification and the repair of DNA damages [1].

The FOXO protein exists in two forms inside the cell. When it is phosphorylated by the AKT factor, FOXO becomes FOXO-P and leaves the nucleus for the cytoplasm, where it loses its properties [2]. Activation of the AKT factor increases with age, thereby provoking a predominance of the inactive form : FOXO-P.

A protein which is able to inhibit AKT activation and therefore maintain FOXO inside the cell nucleus was identified in 1997. Named KLOTHO in reference to the Greek goddess Clotho, who spins the thread of life, it is now considered by scientists to be the NEW YOUTH PROTEIN.

### THE YOUTH PROTEIN KLOTHO

Its mutation causes a premature apparition of all the traditional signs of aging and leads to premature death. On the contrary researchers observed that when it is overexpressed, this gene leads to a 20% to 30% increase in mice lifespan. The Klotho protein is involved in a large number of signaling pathways leading to the re-establishment of optimal cellular function.

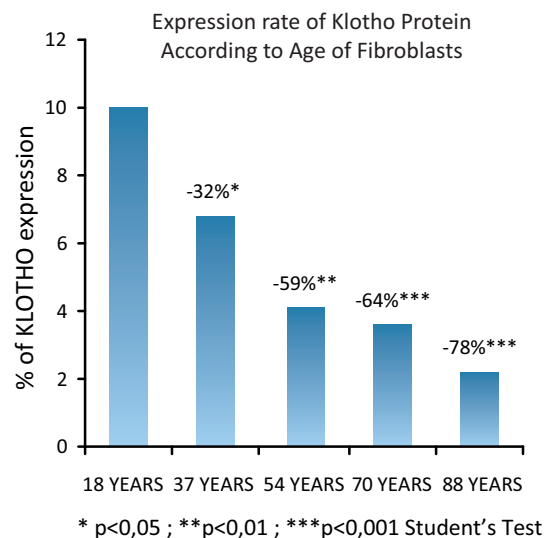
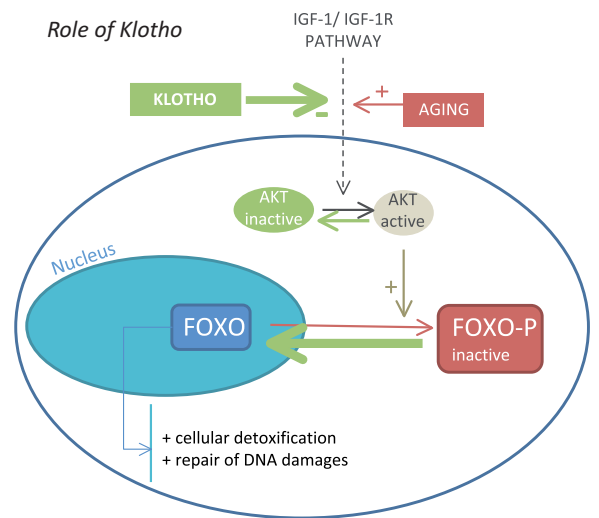
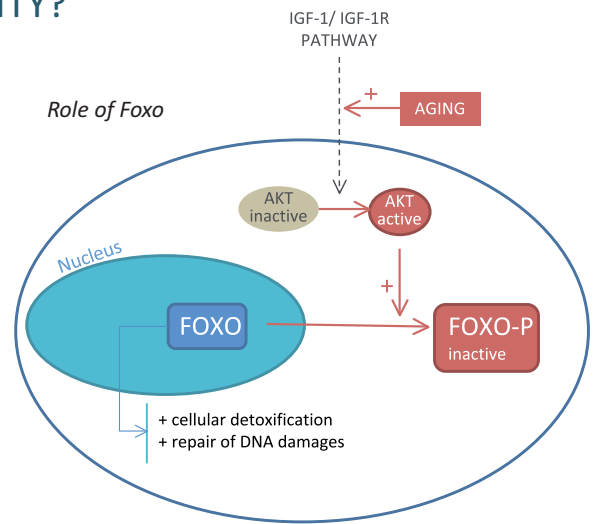
Klotho protein has not yet been studied for cosmetic applications. Our laboratories have shown that its rate of expression is directly linked to the age of human fibroblasts.

#### RESULTS

The decrease in KLOTHO expression starts very early during the ageing process: from roughly 30 years. Then its expression gradually decreases with fibroblasts ageing.

INCREASING KLOTHO SYNTHESIS IN THE FIBROBLASTS THUS REPRESENTS A NEW STRATEGY TO COMBAT SKIN AGING.

*Protocol: human dermal fibroblasts coming from donors of different ages. Quantification of KLOTHO protein by immunofluorescence.*





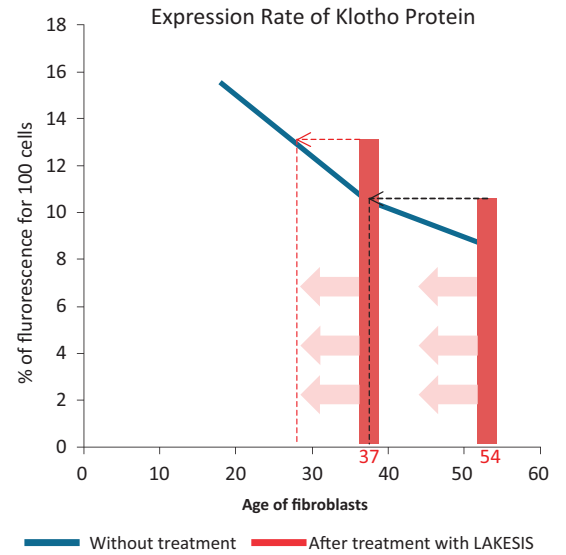
## IN-VITRO TEST ACTIVATION OF YOUTH PROTEINS.

### LAKESIS INCREASES KLOTHO

LAKESIS increases the synthesis of the Klotho protein in fibroblasts, from the earlier to the later stages of ageing process.

After treatment, 54-year-old fibroblasts express the same amount of Klotho as 37-year-old fibroblasts. And 37-year-old fibroblasts express the same amount of Klotho as 28-year-old fibroblasts.

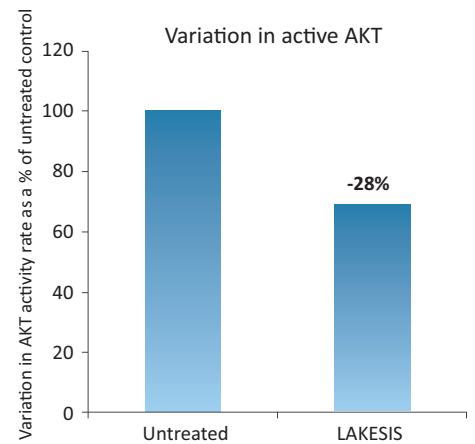
*Protocol: human dermal fibroblasts from donors of different ages and incubated with LAKESIS at 0.005% for 24 hours. Observation and analysis of Klotho expression using immunofluorescence.*



### LAKESIS DECREASES AKT RATE BY 28%

By increasing Klotho synthesis, LAKESIS decreases the level of active AKT.

*Protocol: 54-year-old human dermal fibroblasts treated or not with LAKESIS at 0.005% for 24 hours. Staining and analysis of phosphorylated and non-phosphorylated AKT.*

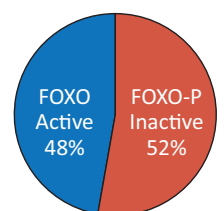


### LAKESIS INCREASES FOXO BY 16%

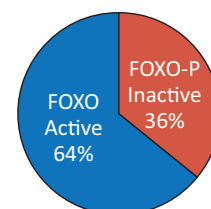
By inhibiting the AKT factor, LAKESIS decreases phosphorylation of the FOXO youth factor. This factor is now mainly located in the nucleus, where it activates the transcription of detoxification and cellular repair factors.

*Protocol: 54-year-old human dermal fibroblasts were treated or not with LAKESIS at 0.005% for 24 hours. Analysis of phosphorylated and non-phosphorylated FOXO.*

Before treatment



After treatment with LAKESIS



LAKESIS REBALANCES THE EFFECTS OF AGEING ON KLOTHO FROM THE EARLY STAGES OF SKIN AGEING. IT THEREFORE PROMOTES THE PREDOMINANCE OF THE ACTIVE FORM OF FOXO AND THUS THE REACTIVATION OF CELLULAR DETOXIFICATION AND GLOBAL ACTIVITY.



## IN-VITRO TEST

### REACTIVATION OF CELLULAR DETOXIFICATION PROCESSES

#### LAKESIS INCREASES THE LEVEL OF ACTIVE CATALASE BY 145%

Catalase is part of the enzymatic pool which detoxifies the cell. By increasing the FOXO factor, LAKESIS reactivates the cellular detoxification processes.

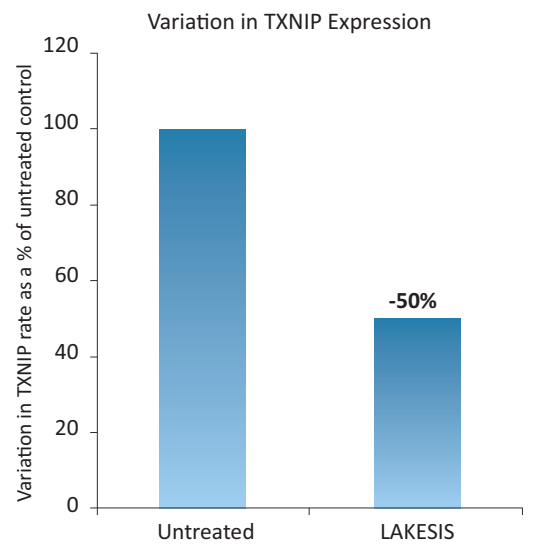
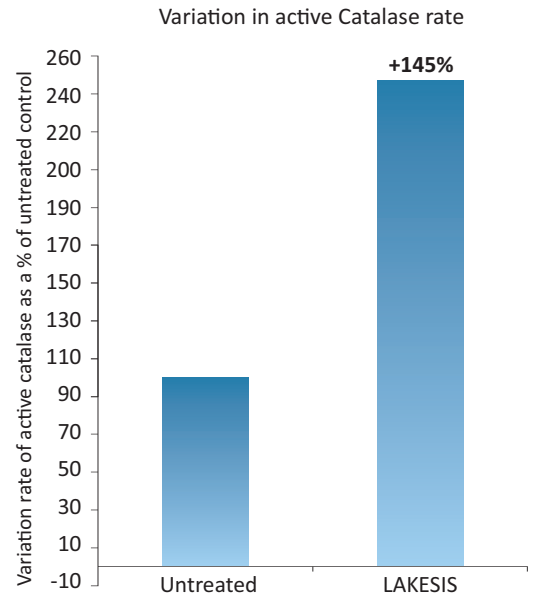
*Protocol: 54-year-old human dermal fibroblasts were treated or not with LAKESIS at 0.005% for 24 hours. Staining and analysis of active catalase.*

#### LAKESIS DECREASES TXNIP BY 50%

Thioredoxin is a universal and very powerful antioxidant system. It has been largely studied mainly for its involvement in combating cellular senescence. It plays a major role in the cellular detoxification process. The **TXNIP factor is a thioredoxin inhibitor**.

By inhibiting TXNIP, LAKESIS promotes cellular detoxification processes.

*Protocol: reconstituted human skin was treated or not with LAKESIS at 0.2% using a topical application for 24 hours. Gene expression analysis was made with mini-chips.*



## IN-VITRO TEST

### LAKESIS IMPROVES CELLULAR ACTIVITY

Gene transcription is the process of reading and translating genetic information in RNA. After transcription, each RNA represents a “recipe” to be used for manufacturing a protein. The amount of RNA present inside a cell provides important information about the rate of cellular activity. With age, it drops.

#### CELLULAR RNA AMOUNT MEASUREMENT

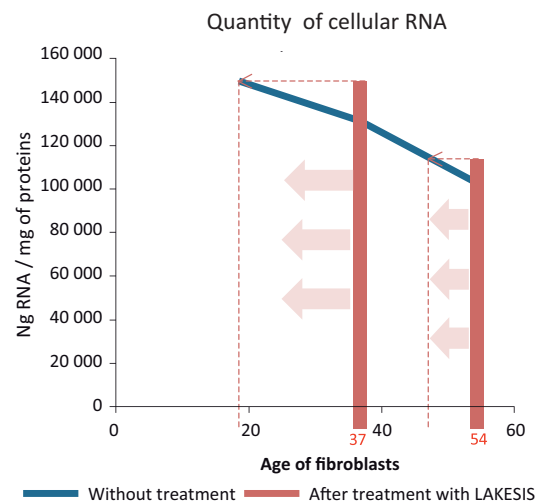
LAKESIS reactivates cellular detoxification. Cells are more active, which is translated here by an increase in the total amount of cellular RNA. 37-year-old fibroblasts achieved a RNA synthesis rate equivalent to that of 18-year-old fibroblasts.

*Protocol: human dermal fibroblasts from donors of different ages. Extraction and quantification of total RNA.*

#### LAKESIS IMPROVES COLLAGEN I SYNTHESIS BY 47%

By relaunching cellular activity, LAKESIS increases collagen I synthesis.

*Protocol: 39-year-old human dermal fibroblasts were treated or not with LAKESIS at 0.003% for 24 hours.*





## IN-VITRO TEST

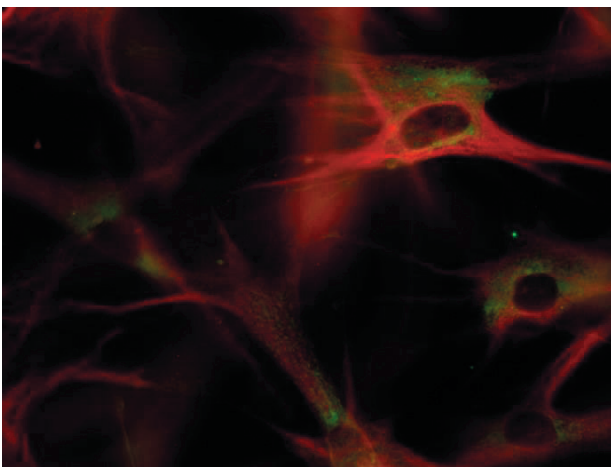
# REJUVENATING EFFECT OF LAKESIS ON THE EXTRA-CELLULAR MATRIX

The use of 3D dermal models enables visualizing fibroblasts in the extracellular matrix and obtaining data on their interaction and activity within the extracellular matrix. We used red staining to visualize the fibroblast cytoskeleton (vimentin) and green staining to visualize synthesis of the proteins we are interested in.

## LAKESIS REACTIVATES FIBRILLIN SYNTHESIS

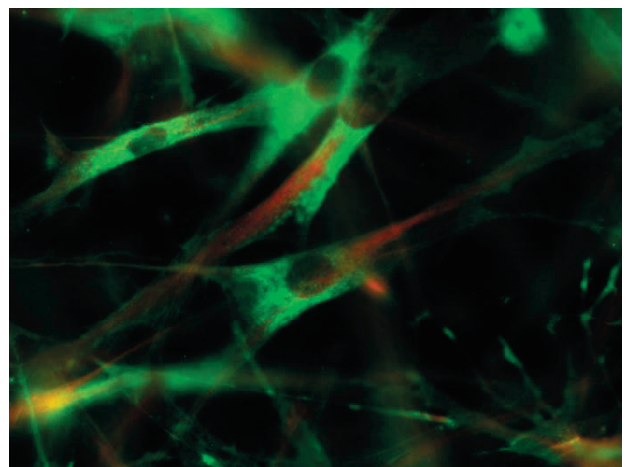
***Fibrillin is a major component of elastic fibers.***

*Protocol: 54-year-old human dermal fibroblasts included in a collagen lattice were treated or not with LAKESIS at 0.005% for five days. Visualization of fibrillin using green immunofluorescence.*



**54-YEAR-OLD FIBROBLASTS**

Visualization of fibrillin synthesis in green.

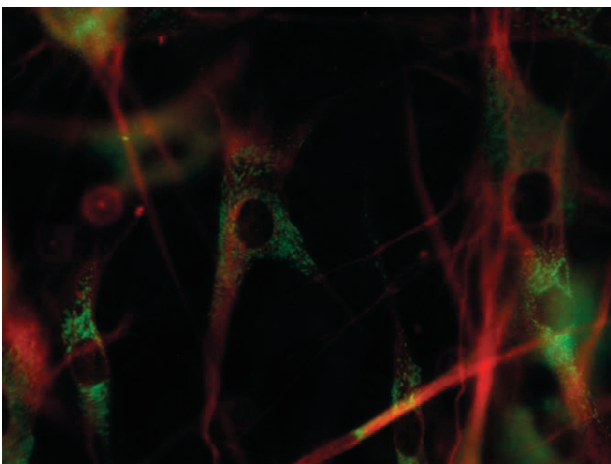


**54-YEAR-OLD FIBROBLASTS TREATED WITH LAKESIS.**

Activation of synthesis and dermis redensification in fibrillin.

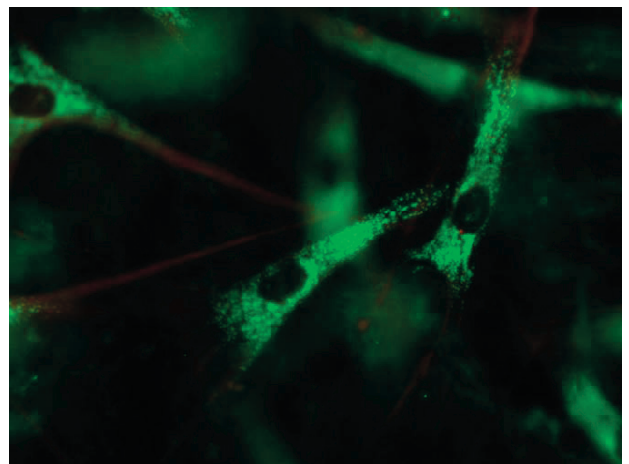
## LAKESIS REACTIVATES COLLAGEN SYNTHESIS

*Protocol: 54-year-old human dermal fibroblasts included in a collagen lattice were treated or not with LAKESIS at 0.005% for five days. Visualization of collagen using green immunofluorescence.*



**54-YEAR-OLD FIBROBLASTS**

Visualization of collagen synthesis in green.



**54-YEAR-OLD FIBROBLASTS TREATED WITH LAKESIS**

Activation of synthesis and dermis redensification in collagen.





## CLINICAL TEST

# ANTI-AGEING EFFECT OF LAKESIS ON DERMIS

### PROTOCOL

20 volunteers aged 55 to 65

Twice-daily application of a cream containing 0.2% LAKESIS for 28 days

Application area: whole face

### DESCRIPTION OF SENEBA – Figure 1.

The SENEBA (SubEpidermal Non-Echogenic Band) is found in the upper layer of the dermis. It is an area very poor in supporting fibers and corresponds to the black band indicated here by pink brackets. Almost non-existent in children, researchers have shown that the thickness of the SENEBA increases with age [4]. The thickening of this fiber-poor area is characterized by a loss of firmness and tissue support.

### EFFECT OF LAKESIS ON SENEBA – Figure 2.

Echography analysis – Supporting fibers in green.

Density of supporting fibers :

+51% on average ( $p < 0.001$ )

And up to +103%

Variation in SENEBA surface

-29% on average ( $p < 0.001$ )

And up to -43%

**LAKESIS stimulates the synthesis of supporting fibers in the SENEBA and decreases the size of this non-echogenic area. This action reinforces the immediate supporting properties of the dermis.**

### EFFECT OF LAKESIS ON THE WHOLE DERMIS – Figure 3.

Echography analysis – Supporting fibers in green.

Homogeneity of supporting fibers distribution:

+16% on average ( $p < 0.01$ )

And up to +34.6%

Variation of supporting fibers density:

+18% on average ( $p < 0.01$ )

And up to +37.4%

**LAKESIS increases supporting fibers density throughout the dermis and also ensures a homogeneous distribution of these fibers.**

Figure 1

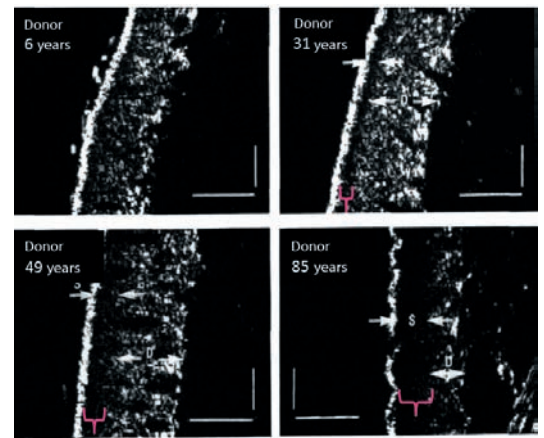


Figure 2

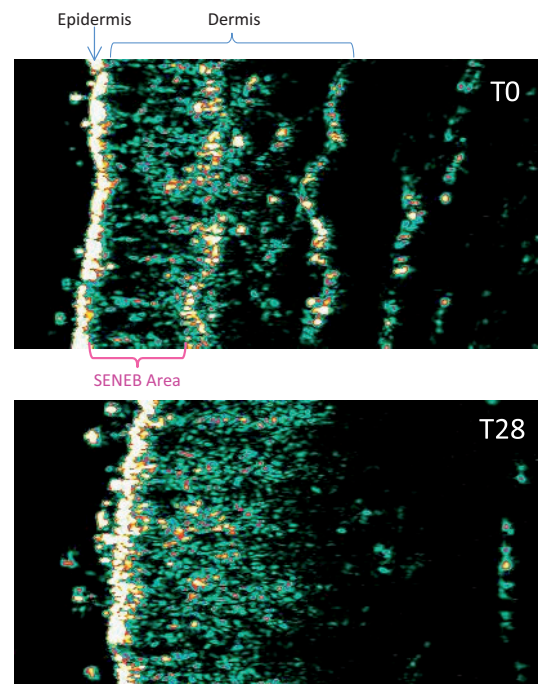
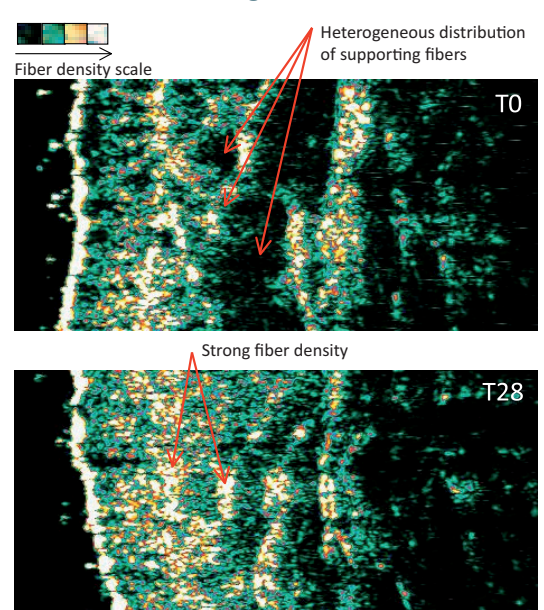


Figure 3







## ANTI-AGEING EFFICACY OF LAKESIS ON THE OVAL FACE

Using fringe projection allows reconstituting the volume of the face at T0 and T28. A superposition of both volumes allows identifying the area where volume has been lifted. Everywhere the skin is yellow means that the volume of these areas has been lifted after 28 days treatment.

Variation of the volume of the face:

-4.41% on average ( $p < 0.05$ ) and up to -16%

2/3 women observed an improvement of facial contours after 4 weeks treatment with LAKESIS.



*Everywhere the skin is yellow means that the volume of these areas has been lifted after 28 days treatment.*



## OVERALL ANTI-AGEING EFFICACY OF LAKESIS

### CLINICAL ANALYSIS OF SKIN YOUTH BY A DERMATOLOGIST:

+8.4% on average ( $p < 0.05$ )

And up to 29%

### CONSUMERS ANALYSIS ON AN ANALOGICAL SCALE:

Facial contour well defined: +24% ( $p < 0.05$ )

Skin firmness: +23% ( $p < 0.1$ )

Skin thickness: +22% ( $p < 0.05$ )

BY AWAKING THE YOUTH PROTEINS KLOTHO AND FOXO, LAKESIS REACTIVATES CELLULAR ACTIVITY TO RE-DENSIFY DERMIS IN COLLAGEN AND FIBRILLIN FIBERS. DERMIS IS RE-DENSIFIED, FACIAL CONTOURS ARE LIFTED, THE SKIN LOOKS THICKER, FIRMER AND YOUNGER.

# FORMULATION GUIDELINES

## LIFT FIRMLING FACE OIL

This FACIAL OIL is formulated with LAKESIS and HYDRASALINOL, a hydra-nourishing active ingredient.

Phase	Raw Material / Trade Name	INCI Name	%
A	DEODORIZED ORGANIC SESAME SEED OIL (1)	Sesamum Indicum Seed Oil	35,15
	VIRGIN ORGANIC CARTHAMUS OIL (1)	Carthamus Tinctorius Seed Oil	8,00
	DEODORIZED SUNFLOWER OIL (1)	Helianthus Annuus Seed Oil	15,00
	CETIOL LC (2)	Coco-Caprylate/Caprata	41,00
	NATROX RO (3)	Helianthus Annuus Seed Oil & Rosmarinus Officinalis Leaf Extract	0,20
	HYDRASALINOL (4)	Caprylic/Capric Triglyceride & Salicornia Herbacea	0,25
	LAKESIS (4)	Caprylic/Capric Triglyceride & Pistacia Lentiscus (Mastic) Gum	0,20
	WATER LILY R18717 FRAGRANCE (5)	Fragrance	0,20

(1) Huilerie Emile Noël, (2) BASF, (3) Naturex, (4) CODIF Recherche et Nature, (5) Parfex

### Protocol:

In an appropriate recipient, add the raw materials and cold mix with a deflocculator at 1000 rpm. Mix for about 10 minutes until a clear solution is obtained.

## **BIBLIOGRAPHIC REFERENCES**

- [1] The multiple roles of FOXO transcription factors. A. Brunet. *Med Sciences*, vol.20, n°10, 2004, p. 856-859.
- [2] Klotho and aging. M. Kuro-o. *BiochemBiophysActa*. 2009 October; 1790(10): 1049-1058.
- [3] <http://www.washingtonpost.com/wp-dyn/content/article/2005/08/25/AR2005082501224.html>
- [4] Jean de Rigal, Catherine Escoffier, (1989). Assessment of aging of the human skin by in vivo ultrasonic imaging. *Journal of investigative dermatology*, 93(5).



# LAKESIS : GLOBAL ANTI-AGEING – YOUTH REACTIVATOR

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## COSMETIC ACTIVITY

Reactivation of youth proteins KLOTTHO and FOXO

Reactivation of cellular detoxification processes

Improvement of cellular activity

Re-densification of the Extra-Cellular Matrix in fibrillin and collagen fibers

Re-densification of dermis

Decrease in SENEK surface

Lifting action on the oval face

Skin looks younger, thicker, and facial contours appear to be redrawn

## INCI NAME

Caprylic/capric triglyceride (and) Pistacia lentiscus (mastic) Gum

RECOMMENDED % OF USE: 0.2%

CHARACTERISTICS: liposoluble active ingredient from vegetal origin – 100% natural

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