Bio Basic Europe

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UNIVERSITA' DEGLI STUDI DI PAVIA
Dipartimento di Biologia e Biotecnologie
"Lazzaro Spallanzani"

Parco Tecnico Scientifico Pavia

BIO BASIC LAB
Vitro and Microbiological Test

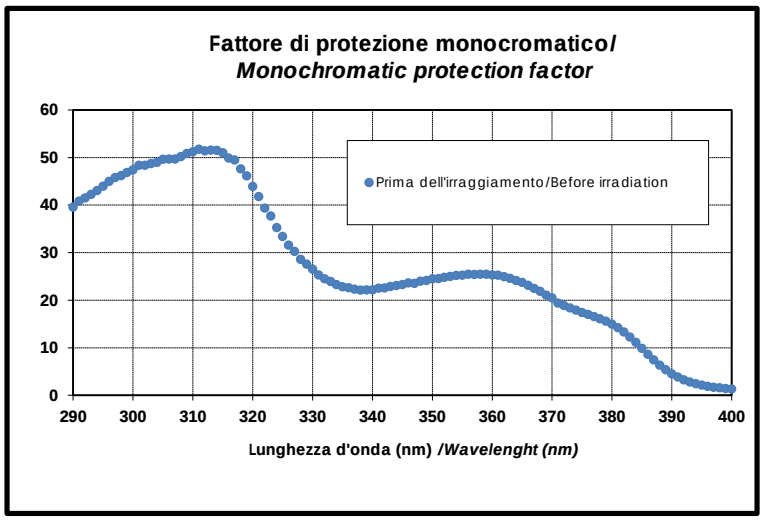
SCREENING TEST:
Determinazione preliminare in vitro della fotoprotezione UVB
Preliminary determination of sunscreen UVB photoprotection in vitro (ISO 24443:2012)

Protocollo n°/*Record no.* **1802E29NP** **PRIMA LUX S.r.l.**
Milano: 23 Luglio 2018/*23rd July 2018*

PRIVILEGE ONE – THE FACE ESSENTIAL

Ingredienti/*Ingredients*

Aqua, Ethylhexyl methoxycinnamate, C12-15 alkyl benzoate, Glyceryl stearate citrate, Butyl methoxydibenzoylmethane, Glycerin, Butyrospermum parkii butter, Diethylhexyl carbonate, Cetyl alcohol, Ethylhexyl triazone, Trehalose, Bulbine frutescens leaf juice, Caprylyl glycol, Dimethicone, Glyceryl stearate, Ethyl ascorbic acid, Hydrolyzed algin, Tocopherol, Hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer, Isohexadecane, Sodium hyaluronate, Xanthan gum, Lecithin, Citric acid, Ascorbyl palmitate, Alpha-isomethyl ionone, 1,2-Hexanediol, Polysorbate 80, Tropolone, Parfum, Citronellol, Disodium EDTA, Geraniol



SPF in vitro	44,4
SPF in vivo^	30

^Etichetta/Labelled

Dr. Claudio Angelinetta
Post-graduate in Cosmetic Science and Technology - University of Milan
Quality Control
Bio Basic Europe S.r.l. – Milano - Italy

Il presente Rapporto di Prova è firmato digitalmente ai sensi della normativa vigente
This test report is digitally signed according to current legislation

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TEST ANTI-POLLUTION - Valutazione dell'azione protettiva *in vitro* di un prodotto cosmetico nei confronti dello stress ambientale mediante determinazione della vitalità cellulare e dosaggio dei radicali liberi

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ANTI-POLUTION TEST - In vitro evaluation of the protective action of a cosmetic product against environmental stress through determination of cell viability and dosage of ROS

PRIMA LUX SRL

SLAB001 - TRATTAMENTO GIORNO ANTIPOLLUTION

Protocollo n° / *Report no.* **1802E31V1**

Luogo e data di emissione MILANO – 31 Luglio 2018
Place and date of issue *MILAN – 31st July 2018*

RIASSUNTO

Il test per valutare l'azione protettiva del prodotto cosmetico nei confronti di agenti inquinanti e ambientali responsabili dell'invecchiamento cutaneo è stato condotto su cheratinociti umani trattati con 3 concentrazioni del prodotto da testare (1.0, 0.5 e 0.1 mg/ml) scelte dopo aver eseguito un test preliminare di citotossicità.
Le cellule sono state quindi stimolate con uno standard che riproduce l'inquinamento ambientale (urban dust), con uno standard di inquinamento indoor o esposte a raggi UVA allo scopo di "mimare" l'esposizione a cui quotidianamente è esposta la pelle. Cellule non trattate rappresentano il controllo negativo.
Si è quindi misurata la vitalità cellulare mediante test MTT e si sono dosati i radicali liberi (ROS) prodotti, confrontando i valori delle cellule non trattate con quelli delle cellule trattate con il prodotto testato.
Si è osservato nelle cellule irraggiate e trattate con il campione testato alla concentrazione di 1.0 mg/ml la vitalità è superiore rispetto alle cellule irraggiate e non trattate. Nelle cellule irraggiate e trattate con campione a tutte le concentrazioni testate i livelli di ROS sono inferiori rispetto alle cellule irraggiate e non trattate.
Si può pertanto concludere che il prodotto testato ha un'attività "anti-pollution" che si manifesta in particolar modo nei confronti dell'irraggiamento UV.

ABSTRACT

The tests to evaluate the protective action of the cosmetic product against pollutants and environmental agents responsible for skin aging was conducted on human keratinocytes treated with 3 concentrations of the tested product (1.0, 0.5 and 0.1 mg/ml) chosen after a preliminary cytotoxicity test.
The cells were stimulated with a standard of atmospheric pollution (urban dust), with a standard of indoor pollution or exposed to UVA rays in order to "mimic" the exposure to which the skin is daily exposed. Untreated cells represent the negative control.
We measured the cell viability (through an MTT assay) and ROS production comparing the values of untreated cells with the one of cells treated with the tested product.
We observed that in the cells irradiated and treated with the tested sample at the concentration of 1.0 mg/ml the viability is higher compared to the irradiated and untreated cells. In the cells irradiated and treated with the sample at all tested concentrations, ROS levels are lower than in irradiated and untreated cells.
We can therefore conclude that the tested product has an "anti-pollution" activity, which is manifested in particular with regard to UV rays.

MATERIALI E METODI

MATERIALS AND METHODS

Colture cellulari

Il test è stato condotto su cheratinociti umani (Huker) coltivati in DMEM (Dulbecco's modified Eagle medium) contenente 10% di siero fetale bovino (FBS) e 1% di antibiotici (penicillina e streptomicina) ed incubati in condizioni di coltura standard (37°C, 5% CO_2). Sono state applicate le buone pratiche per la coltivazione di cellule.

Cell line and culture conditions

The test was performed on human keratinocytes (Huker) cultured in DMEM (Dulbecco's modified Eagle medium) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin) and incubated at standard culture conditions (37°C, 5% CO_2). Good cell culture practices were used.

Campione testato / *Tested sample*

SLAB001 - TRATTAMENTO GIORNO ANTIPOLLUTION

INCI

Aqua, Ethylhexyl methoxycinnamate, C12-15 alkyl benzoate, Glyceryl stearate citrate, Butyl methoxydibenzoylmethane, Glycerin, Butyrospermum parkii butter, Diethylhexyl carbonate, Cetyl alcohol, Ethylhexyl triazone, Trehalose, Bulbine frutescens leaf juice, Caprylyl glycol, Dimethicone, Glyceryl stearate, Ethyl ascorbic acid, Hydrolyzed algin, Tocopherol, Hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer, Isohexadecane, Sodium hyaluronate, Xanthan gum, Lecithin, Citric acid, Ascorbyl palmitate, Alpha-isomethyl ionone, 1,2-Hexanediol, Polysorbate 80, Tropolone, Parfum, Citronellol, Disodium EDTA, Geraniol.

Conditions of stimulation

In order to simulate a condition of environmental stress, the cells were stimulated with a standard of urban pollution (urban dust), cigarette smoke and subjected to irradiation with UVA rays.

1. ***Urban dust.*** *The urban dust is a standard reference material containing polyciclic aromatic hydrocarbons (PAHs), nitro-substituted PAHs, polychlorinated biphenyl (PCB), chlorinated pesticides and inorganic heavy metals. The standard was prepared from atmosferic particulate material collected in the Washington DC area over a period longer than 12 months using a baghouse specially designed for the purpose. The urban dust standard is not intended to be representative of the area in which it was collected, but it should generally tipify atmospheric particulate matter obtained from an urban area.*
2. ***Indoor standard.*** *The indoor standard is a mixture of 52 components achieved analizying the vacuum cleaner bags obtained from households, cleaning services, motels, and hotels from North Carolina, Maryland, Ohio, and New Jersey.*
3. ***UV rays.*** *The lamp used in the experiments is a solar light simulator. A constant emission in the UVA range (315-400 nm) with an irradiance of 1.7 mW/cm^2 is assured. The UVB emission is screened in order to avoid direct cell mortality.*

Test execution

We performed a preliminary MTT test in order to choose non-cytotoxic concentrations of tested product to be used: the cells were treated with several dilutions of the tested sample. Untreated cells maintained in growth medium represent the negative control. After 24 hours of contact, the cells were washed with phosphate buffered saline (PBS) and subjected to MTT assay.

For the "anti-pollution" test, an adequate number of cells was seeded in 96-well plates. Once reached a semi-confluent monolayer, the cells were treated with the chosen concentrations of the tested sample and different stimuli able to reproduce the skin exposure to atmospheric pollutants. Intensity stimuli were chosen in order to cause significant cell damage (reduction of viability by at least 20-30%). Untreated cells kept in culture medium represent negative controls. At the end of an overnight contact period the cells were washed with a phosphate buffer (phosphate buffer saline, PBS) in order to eliminate any treatment residues and then subjected to MTT tests to measure residual viability and to the dosage of ROS with DCFA.

CONCLUSIONI

Il campione denominato

SLAB001 - TRATTAMENTO GIORNO ANTIPOLLUTION

ha azione protettiva nei confronti dello stress ambientale su cheratinociti *in vitro*.
Tale azione di manifesta in particolare nei confronti dell'irraggiamento UV

CONCLUSIONS

The sample called

SLAB001 - TRATTAMENTO GIORNO ANTIPOLLUTION

has protective action against environmental stress on keratinocytes in vitro.
This action is particularly evident with regard to UV rays

UNIVERSITA' DEGLI STUDI DI PAVIA
DIPARTIMENTO DI MEDICINA INTERNA E TERAPIA MEDICA
(Direttore: Prof. Plinio Richelmi)

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CDC Istituto di Ricerche Dermo-Cliniche

Valutazione dell'effetto e della gradevolezza di un prodotto cosmetico mediante test clinico

Evaluation of the effect and of the acceptability of a cosmetic product through a clinical test

PRIVILEGE ONE CONCENTRATE GREAT THE FACE ESSENTIAL
lotto 260618

PRIMA LUX SRL

Protocollo n° / *Report no.* **1801E31F**

Luogo e data di emissione: MILANO – 24 Agosto 2018
Place and date of issue: MILAN – 24th August 2018

SOMMARIO

Il prodotto cosmetico oggetto del presente test è stato sottoposto ad indagine clinica per valutare se possieda effetto nel migliorare l'elasticità, la compattezza, la luminosità e la levigatezza cutanea.
Inoltre ne è stata valutata la gradevolezza all'uso.
Il test è stato condotto da un dermatologo membro dello staff Bio Basic Europe ed è così articolato: sono state selezionate 20 volontarie aventi età compresa tra i 16 ed i 60 pelle poco tonica e incarnato spento, cui è stato chiesto di applicare il prodotto in oggetto sul viso, due volte al giorno per 28 giorni consecutivi. Durante questo periodo sono state valutate strumentalmente l'elasticità e la luminosità cutanea e clinicamente la compattezza e la levigatezza cutanea.
Alla fine del test sono state poi raccolte una serie di valutazioni sensoriali espresse dalle stesse volontarie. Per l'autovalutazione si è utilizzata la scala VNS con valori da 0 a 10.
In base ai risultati ottenuti possiamo affermare che il prodotto cosmetico testato ha dimostrato possedere un effetto nel migliorare l'elasticità, la compattezza, la luminosità e la levigatezza cutanea nelle volontarie sottoposte a test clinico.
Il prodotto ha inoltre dimostrato possedere una buona gradevolezza all'uso.

SUMMARY

The purpose of this clinical test is to evaluate if the tested cosmetic product has an effect in improving skin elasticity, compactness, lightness and smoothness. Its acceptability to use is also evaluated.
This test was performed by a dermatologist member of Bio Basic Europe staff: 20 female panellists, with an age between 18 and 60 years and with low toned skin and dull skin complexion were recruited and were asked to apply the cosmetic product on the face, twice per day for 28 consecutive days. During this period, skin elasticity and skin lightness have been instrumentally evaluated and skin compactness and smoothness have been clinically evaluated.
All the evaluations given by volunteers in the sensorial test were collected at the end of this test.
The score they gave is according to VNS scale (0-10 where 0 is the minimum value and 10 is the maximum one).
According to the obtained results we can state that, in the volunteers who underwent the clinical test, the tested product has proved to have an effect in improving skin elasticity, compactness, lightness and smoothness.
The product has also proved to have a good acceptability.

PARTE SPERIMENTALE

EXPERIMENTAL PART

Protocollo n° 1801E31F

Report no. 1801E31F

Titolo

Valutazione dell'effetto e della gradevolezza di un prodotto cosmetico mediante test clinico

Title

Evaluation of the effect and of the acceptability of a cosmetic product through a clinical test

Scopo

Il test consente di valutare se il prodotto cosmetico sottoposto a tale test possieda un effetto nel migliorare l'elasticità, la compattezza, la luminosità e la levigatezza cutanea.

Il test fornisce inoltre informazioni sulla gradevolezza d'uso del prodotto.

Aim

The purpose of this test is to evaluate whether the tested cosmetic product has an effect in improving skin elasticity, compactness, lightness and smoothness.

Product acceptability is evaluated too.

Informazioni legali

In accordo alla normativa vigente, e alla dichiarazione di Helsinki, i volontari sono adeguatamente informati circa lo scopo, le modalità e le caratteristiche dello studio clinico, gli effetti favorevoli ed i possibili effetti collaterali. Ciascun volontario firma per accettazione un modulo di consenso informato, gestito ed archiviato in accordo alle procedure interne del Sistema Gestione Qualità di Bio Basic Europe S.r.l.

Legal information

In accordance with the current legislation and the declaration of Helsinki, all volunteers must be adequately informed of the aims, methods, clinical trial details, anticipated benefits and potential undesirable effects of the study. Each panellist must sign an informed consent form, which is managed and archived by applying the internal procedure of the Quality Management System of Bio Basic Europe S.r.l.

Informazioni contrattuali

- Relazione tecnica eseguita come da contratto tra BIO BASIC EUROPE S.r.l. ed Università degli Studi di Pavia.
- Stesura del report eseguita presso BIO BASIC EUROPE S.r.l. per conto di PRIMA LUX SRL
- Sperimentazione eseguita presso CDC - Istituto di Ricerche Dermo-Cliniche

Contract information

- *Technical report performed by BIO BASIC EUROPE S.r.l. and Università degli Studi di Pavia.*
- *Final technical report written by BIO BASIC EUROPE S.r.l. on behalf of PRIMA LUX SRL*
- *Experimentation performed at CDC - Dermo-clinic Research Institute*

CARATTERISTICHE DELLO STUDIO

Soggetti del test

Sono stati selezionati 20 soggetti di sesso femminile, aventi età compresa tra i 18 ed i 60 anni, secondo i seguenti criteri di inclusione:

- pelle poco tonica
- incarnato spento

- Buono stato di salute generale/assenza di disturbi psicologici e/o cognitivi;
- Assenza di patologie dermatologiche ed allergologiche (cosmetologiche o ad altri eccipienti specifici), o altre patologie (tipo reazione irritative di origine non nota);
- Assenza di trattamenti farmacologici in atto che possano influire sull'esito del test;
- Non partecipazione ad altri studi clinici nei 30 giorni precedenti;
- Ottenimento del consenso informato.

Preparazione dei campioni

I campioni sono stati applicati, in funzione delle loro caratteristiche d'uso: tal quale.

Metodo di applicazione dei campioni

I campioni sono stati applicati sul viso, due volte al giorno per 28 giorni consecutivi.

CLINICAL TEST FEATURES

Test subjects

20 female subjects, with an age between 18 and 60 years, have been selected for the test, following the undermentioned inclusion criteria:

- *low toned skin*
- *dull skin complexion*

- good state of health/absence of psychological and/or cognitive disorders;
- no dermatopathies and allergic pathologies (to cosmetics or other specific excipient), or other pathologies (as unknown irritant responses);
- no ongoing pharmacological treatments that could affect the result of the test;
- no participations in other clinical trial during the previous 30 days;
- signature of the informed consent form.

Preparation of the samples

Samples of the products have been applied following their usual use: as they are.

Method of application of the samples

Samples of the tested product have been applied on the face, twice per day for 28 consecutive days.

EXECUTION OF THE TEST

During the test, the following parameters have been evaluated:

INSTRUMENTAL PARAMETERS

*- Skin **elasticity** measurements are performed by using the elastometer CUTOMETER® – MPA 580*

Skin elasticity was measured with Cutometer®. Before the test, it was necessary to standardize the measuring technique, of the level (maximum 500 mBar) and of the time of suction, with subsequent release, as well as the number of repeated measurements in the same test area (maximum 9). For this research, a suction cycle of 1 second at 500 mBar followed by a releasing cycle of one second was selected.
Regarding the elastometric measurement, the skin surface was aspirated from the depression induced by the machine into the aperture of the elastometer's measuring probe.
Depth of skin penetration inside the probe was measured by an optic sensor.
Linkage to a PC allowed the data obtained to be displayed, stored and printed. Cutaneous elasticity reflects the skin's potential capacity (measured in mm) for retraction.
A graph shows the deformation curve of skin undergoing aspiration, and includes two components (see the figure).

- *An elastic component (Ue), which corresponds to the part of the curve that rises rapidly, and is reversible to the deformation;*
- *A plastic component (Uv), which corresponds to the part of the curve that rises slowly and is not completely reversible to the deformation.*

❖ *During the releasing phase, the quantity of deformation remaining in the skin can be observed (Ua-Ur=residual deformation).*
Cutaneous elasticity is defined as the ratio:

Elasticity= Ua/Uf (R2)

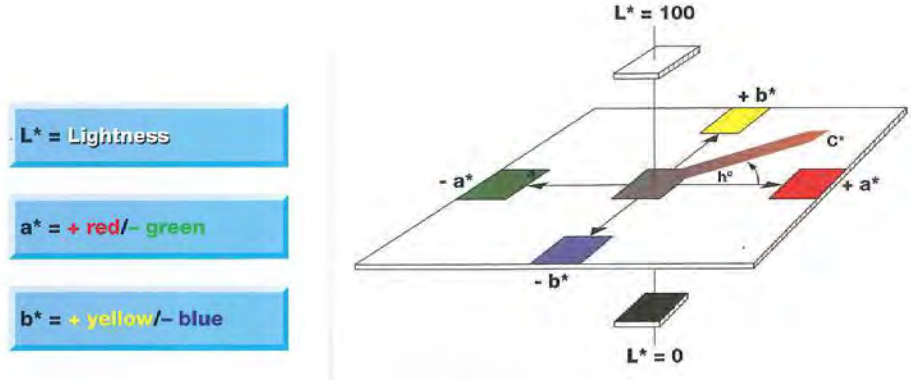
Which represents the recovery degree of the maximum deformation reached, whose values range between 0 and 1 (maximum elasticity).

*- Skin **lightness** measurements are taken by using SPECTRO GUIDE CIE L*a*b* system sphere GLOSS BYK – GARDNER** and then analysed by using EASY-LINK software*

***portable spectrophotometer: measurement principle is based on the measurement of spectral reflectance within the visible spectrum of wavelengths from 400-700nm.*

*The colour is expressed in the L*a*b* space as defined by the Commission Internationale de l'Enclairage (CIE) in 1976*

*In particular we evaluate L * value that indicates the Lightness.*



CLINICAL PARAMETERS

Evaluations performed:

- skin smoothness
- skin compactness

The readings and the evaluations have been performed:

- *at [t0] (basal value)*
- *after product use: after 14 days [t14] and after 28 days [t28]*

SELF-EVALUATIONS

Volunteers opinions are also taken after product use: after 28 days [t28].
This self-evaluation was performed according to VNS scale where 0 is the minimum value and 10 is the maximum value.

EVALUATION AND RECKONING OF THE INSTRUMENTAL RESULTS

The statistical analysis has been performed using ***Paired t-test****: we decided to fix the threshold of acceptability at 1% .*

EVALUATION OF CLINICAL PARAMETRS

The statistical analysis has been performed using the Wilcoxon test: we decided to fix the threshold of acceptability at 5%.

To carry out a statistical survey and to be able to evaluate the skin variations in a specific period, the following skin parameters have been analysed:

Skin compactness ***Skin smoothness***	
Skin is not compact/ Skin is not smooth	*Insufficient*
Skin is little compact/ Skin is little smooth	*Sufficient*
Skin is fairly compact/ Skin is fairly smooth	*Fairly good*
Skin is compact/ Skin is smooth	*Good*
Skin is very compact/ Skin is very smooth	*Very Good*

CONCLUSIONI

In base ai risultati ottenuti possiamo affermare che il prodotto cosmetico:

CONCLUSIONS

According to the obtained results we can state that the cosmetic product:

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lotto 260618

ha dimostrato possedere un effetto nel migliorare l'elasticità, la compattezza, la luminosità e la levigatezza cutanea nelle volontarie sottoposte a test clinico.
Il prodotto ha inoltre dimostrato possedere una buona gradevolezza all'uso.

In the volunteers who underwent the clinical test, has proved to have an effect in improving skin elasticity, compactness, lightness and smoothness.
The product has also proved to have a good acceptability.

Sperimentatore */ Experimenter*
Dott. Fernando Marco BIANCHI

Monitor
Prof. Plinio RICHELMI

Controllo Qualità / *Quality Control*
Dott. Claudio ANGELINETTA