TITLE OF THE STUDY

IN VITRO UVA PF EVALUATION WITH PRE-IRRADIATION
IN VITRO CRITICAL WAVELENGTH EVALUATION WITH PRE-IRRADIATION
ACCORDING TO PN-EN ISO 24443:2021 METHOD

EUROFINS DERMSCAN POLAND STUDY NUMBER

23E1507-3PL

TEST ITEM

SPF 50 Luminous Balm, MRX071-1 – B/N 02125

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1. SUMMARY

Name and address of Sponsor	**PELHAM GROUP** Unit 2, 3 & 4A Old Station Yard Station Road, Petworth, West Sussex GU28 OJF UNITED KINGDOM
Name and address of test facility	**Eurofins Dermscan Poland** Ul. Matuszewskiego 12 80 - 288 GDANSK POLAND Telephone: + 48 58 732 02 90 www.dermscan.pl
Name and address of study director	**Artur Grześkiewicz** Ul. Matuszewskiego 12 80 - 288 GDANSK POLAND Telephone: + 48 58 732 02 90 www.dermscan.pl
Study initiation date (yyyy.mm.dd)	03.07.2023
Study completion date (yyyy.mm.dd)	03.07.2023
Experimentation date (yyyy.mm.dd)	03.07.2023
Estimate number	23D1507
Eurofins Dermscan France study number	23E1507
Dermscan Poland study number	23E1507-3PL
Dermscan test item number	23P1506-3PL
Test item identification	SPF 50 Luminous Balm, MRX071-1 – B/N 02125
Reference on the sample (if different)	NA
Formula name	NA
Batch number	NA
SPF in vivo (ISO 24444:2019)	**Full study (10 subjects) 62,4**
Results	**UVApf 12,9** **Critical Wavelength [nm] 370**

2. PURPOSE OF THE STUDY

The purpose of this study is to determine the in vitro UVA protection factor of a sun protection against UVA radiation (UVAPF), which can be derived mathematically with in vitro spectral modelling, by an in vitro test, using UV Spectrophotometer, in accordance to ISO 24443 method.

3. PRINCIPLE OF THE STUDY

The test is based on the assessment of UV-transmittance through a thin film of sunscreen sample spread on a roughened substrate, before and after exposure to a controlled dose of radiation from a defined UV exposure source. Because of the several variables that cannot be controlled with typical thin film spectroscopic techniques, each set of sunscreen transmission data is mathematically adjusted so that the in vitro SPF data yield the same measured in vivo SPF value that was determined by in vivo testing. Samples are then exposed to a specific measured dose of UV radiation to account for the photostability characteristics of the test item. The resulting spectral absorbance data have been shown to be useful representation of both the width and height of the UVA protection characteristics of the sunscreen item being tested. The mathematical modelling procedure has been empirically derived to correlate with human in vivo (persistent pigment darkening) test results.

4. REFERENCE DOCUMENTS

European Standard, Determination of sunscreen UVA photoprotection in vitro (PN-EN ISO 24443:2021)

5. MEDIA AND REAGENTS

Name of the reagent:	Producer/ Supplier:	Expiration date:
Glycerin	CHEMPUR	06.2025

6. CONSUMABLES

Name:	Model	Manufacturer
Polymethylmethacrylate (PMMA) plates	Moulded - HD6	Helioscreen
Polymethylmethacrylate (PMMA) plates	Sandblasted - SB6	Helioscreen
Tips for multipipette	Combitips advanced	Eppendorf

** NA – not applicable*

7. EQUIPMENTS

Name:	Manufacturer	Serial number
Multipette® M4	Eppendorf	J40533K
XA 220.4Y Analytical scale	RADWAG	553611
UV2000S UV transmittance analyzer	Labsphere	925178650
SOLAR SIMULATOR LS1000-4S-009	SOLAR LIGHT	27305

8. PREPARATIONS

8.1 Absorption measurements through the plate

Prepare a “blank” plate by spreading 15 microliters of glycerin on the roughened side of the plate. Any excess of glycerin should be avoided. Measure the absorbance through this “blank” plate and use this as the baseline measurement for subsequent measurements.

8.2 Sample application

The sunscreen product is applied to a new untreated roughened PMMA plate (with the roughened side uppermost) by mass, at an application rate of 1,3 mg/cm2 (±1,6 %) for moulded plates and 1,2 mg/cm2 (±1,5 %) for sandblasted plates.

To ensure dose accuracy and repeatability, the application area should be not less than 16 cm2.
The application dose may be determined by measuring the mass loss of the pipette before and after application of the product; alternatively, it may be applied based on volumetric measurements with consideration of the specific gravity of the test sample.

Where possible, a positive-displacement automatic pipette should be used for this purpose.
Plates should be weighed after application phase for any non-volatile product.
The sunscreen is applied as at least twelve small droplets of approximate equal volume, distributed evenly over the whole surface of the plate.

Finger cots should not be used to spread the product on the plate.
The fingertip used for spreading shall be dipped into the test product and then wiped to remove excess product before spreading the test product applied to the plate. The fingertip used to spread the product shall be cleaned between applications of different test products.

Deposit and weighing shall not take more than 30 s.

The first test plate applied should not be used for all the measurements, but to adjust the quantity.
After the sunscreen product is deposited on the surface of the plate, it shall be spread immediately over the whole surface using light strokes with human fingertip or mechanical fingertip.

Spreading should be completed in a two-phase process:

First, the product should be spread on the whole area of the plate, using circular movements with a minimum of four passages from the top to the bottom of the plate. At the end of the first pass, a turn of the plate has to be done (¼ turn) to alternate passages, with minimal pressure and repeat this movement three times at least (about 30 s).

Then the sample should be rubbed on the plate surface using alternating horizontal and vertical strokes repeated at least three times alternate passages with a moderate but increased pressure. The second phase should last about 30 s with
For alcoholic or oil products, application should be adapted as follows:
First, the product should be spread on the whole area of the plate, using circular movements with a minimum of three passages from the top to the bottom of the plate. At the end of the first pass, a turn of the plate has to be done (¼ turn) to alternate passages, with minimal pressure and repeat this movement two times at least (about 20 s to 25 s).

Then the sample should be rubbed on the plate surface using alternating horizontal and vertical strokes repeated at least two times alternate passages with a moderate but increased pressure. The second phase should last about 20s with increased moderate pressure.

For all kinds of products, the treated sample shall be allowed to dry for 30 min to 60 min in the dark at the same temperature under UV exposure conditions (i.e. if UV source exposure conditions will be 30 °C, then the drying conditions should also be at 30 °C; or if the UV source exposure conditions will be 27 °C, then the drying conditions should also be 27 °C).
Spray products provided in a pressurized container shall first be degassed by puncturing a very small pinhole in the container to relieve all of the pressure, and then allowed to rest for at least 24 h at room temperature before accessing the liquid for testing.

8.3 Absorbance measurements of the test item-treated plate

The test item-treated plate is placed in the light-path of the UV spectrophotometer and the absorbance of UV radiation through the sample is determined for each wavelength, from 290nm to 400nm, in 1nm steps. One or more observations of absorbance may be made per plate and the mean value shall be determined for each plate.

8.4 Number of determinations

At least four plates prepared with the test sunscreen shall be used to establish the protection aspects of the test sample. Additional plates shall be added to the sampling if the 95% confidence interval (C) is greater than 17% of the mean value of the UVAPF value, until the 95% CI is less than 17 % of the mean UVAPF value.

9. LIGHT SOURCE

The spectral irradiance at the exposure plane of the artificial UV source, that was used for irradiation, was as similar as possible to the irradiance at ground level under a standard zenith sun, as defined by COLIPA (1994) or in DIN 67501 (1999). The UV irradiance was within the following acceptance limits (measured at sample distance).

Total UV irradiance (290 to 400 nm)	40 – 200 W/m2
Irradiance ratio of UVA(320 to 400 nm) to UVB(290 to 320 nm)	11-22

10. EXPERIMENTAL CHRONOLOGY

1. Conduct the calibration and validation of the test equipments, including the UV spectrophotometer used for transmission/ absorbance measurements and the UVA radiometer used to measure the UV exposure source, and verify the transmission properties of the test plates.

2. Conduct blank measurements of a glycerin-treated plate for the reference “blank”, which will be used in the subsequent absorbance measurements.

3. Conduct in vitro absorbance measurements of the sunscreen item spread on a PMMA plate, prior the any UV irradiation. Acquire the initial UV absorbance spectrum with A0(λ) data, where A0(λ) is the mean monochromatic absorbance of the test item layer before UV exposure.

4. Conduct the mathematical adjustment of the initial UV absorbance spectrum to achieve an in vitro SPF (no UV dose) equal to the in vivo SPF. Initial $UVAPF_0$ is calculated - the in vitro UVA Protection Factor measured before sample UV exposure. It is derived from the transmittance curve of the unexposed sample, weighted with the PPD action spectrum and with the “standard” output spectrum of a UVA-filtered solar simulator, after adjustment to the labeled SPF.

5. A single UV exposure dose, D, is calculated, equal to 1,2 x $UVAPF_0$ in J/cm2.

6. Conduct UV exposure of the same sample, according to the calculated UV exposure dose D.

7. Measure the in vitro absorbance of the sunscreen item after UV exposure. Acquire the second UV spectrum with A(λ) data.

8. Conduct the mathematical adjustment of the second absorbance spectrum (following UV exposure). The resulting absorbance curve is the final adjusted absorbance values.

11. METHOD OF CALCULATION

UVA PF Calculation

Calculation of the in vitro SPF:

The in vitro SPF is calculated using the following formula:

$$\text{In vitro SPF} = \frac{\int_{290\text{ nm}}^{400\text{ nm}} \mathrm{E}(\lambda), \mathrm{I}(\lambda), \mathrm{d}(\lambda)}{\int_{290\text{ nm}}^{400\text{ nm}} \mathrm{E}(\lambda), \mathrm{I}(\lambda), \mathrm{T}(\lambda), \mathrm{d}(\lambda)}$$

Where:

E(λ) = Erythema action spectrum (CIE-1987) (see Annex C of ISO 24443);

I(λ) = Spectral irradiance received from the UV source (SSR for SPF testing) (see Annex C of ISO 24443);

A0(λ) = Mean monochromatic absorbance of the test item layer before UV exposure;

D(λ) = wavelength step (1nm)

Calculation of the adjusted in vitro SPF and determination of the coefficient of adjustment C:

C is the coefficient of adjustment, iteratively determined to adjust the calculated in vitro SPF value to the measured (in vivo) SPF value. It is recommended that C falls within a range between 0.6 and 1.6.

$$\text{In vitro SPF adj} = \text{SPF in vivo} = \frac{\int_{290\text{ nm}}^{400\text{ nm}} \mathrm{E}(\lambda), \mathrm{I}(\lambda), \mathrm{d}(\lambda)}{\int_{290\text{ nm}}^{400\text{ nm}} \mathrm{E}(\lambda), \mathrm{I}(\lambda), 10^{-\mathrm{A0}(\lambda),\mathrm{C}}, \mathrm{d}(\lambda)}$$

Calculation of UVA PF0:

$UVAPF_0$ is calculated for each plate individually, according to formula:

$$\text{UVA PF}_0 = \frac{\int_{320\text{ nm}}^{400\text{ nm}} \mathrm{P}(\lambda), \mathrm{I}(\lambda), \mathrm{d}(\lambda)}{\int_{320\text{ nm}}^{400\text{ nm}} \mathrm{P}(\lambda), \mathrm{I}(\lambda), 10^{-\mathrm{A0}(\lambda),\mathrm{C}}, \mathrm{d}(\lambda)}$$

Where:

P(λ) = Persistent pigment darkening (PPD) action spectrum (see Annex C of PN-EN ISO 24443:2021);

I(λ) = Spectral irradiance received from the UVA source (UVA 320-400nm for PPD testing) (see Annex C of PN-EN ISO 24443:2021);

A0(λ) = mean monochromatic absorbance of the test item layer before UV exposure;

D(λ) = wavelength step (1nm)

Calculation of UVA PF of plates after UV irradiation of the sample

The in vitro UVA PF is calculated using the following formula:

$$\text{UVA PF} = \frac{\int_{320\text{ nm}}^{400\text{ nm}} \mathrm{P}(\lambda), \mathrm{I}(\lambda), \mathrm{d}(\lambda)}{\int_{320\text{ nm}}^{400\text{ nm}} \mathrm{P}(\lambda), \mathrm{I}(\lambda), 10^{-\mathrm{A0}(\lambda),\mathrm{C}}, \mathrm{d}(\lambda)}$$

Where:

A(λ) is the mean monochromatic absorbance of the test item layer after UV exposure.

Mean UVA PF0 and UVA PF calculation

Each plate has been measured at least at 5 different sites to ensure that a total area of at least 2 cm2 was measured.

UVA PF_0 or UVA PF of one plate was calculated from the mean absorbance value from the 5 individual spots.

UVA PF_0 or UVA PF of the item is the mean of the UVA PF_0 or UVA PF´s of 4 individual plates.

Calculation of Critical Wavelength (λc)

The critical wavelength (λc) is calculated according to formula:

$$\int_{290\ \mathrm{nm}}^{\lambda c} A(\lambda) \times \delta(\lambda) = 0.9 \times \int_{290\ \mathrm{nm}}^{400\ \mathrm{nm}} A(\lambda) \times \delta(\lambda)$$

Where:

A(λ) is the mean monochromatic absorbance of the test item layer after UV exposure.

The λc of the test item is obtained by the calculation of the arithmetical average of the different measures: all tests corresponding to selected samples are taken into account for the calculation of the statistical dispersion.

12. DEVIATIONS

None

13. RESULTS/CONCLUSION

SPF 50 Luminous Balm, MRX071-1 – B/N 02125		Date of test
UVAPF$_0$ value	12,9	03.07.2023
Irradiation Dose [J/cm^2]	15,5	
UVAPF mean value	**12,9**	
Critical Wavelength mean value	**370**	
C' Coefficient mean value	0,81	
Substrate	HD6 Moulded	
S2 reference		**Date of test**
UVAPF mean value	12,8	22.06.2023
Critical Wavelength mean value	378	
P8 reference		**Date of test**
UVAPF mean value	19,1	22.06.2023
Critical Wavelength mean value	381	

This report relates only to the test item provided by the sponsor and submitted to this study.

14. SAMPLES STORAGE AND ARCHIVING

The test item will be kept for 6 months: At room temperature according to our procedures (P-23).

After 6 months, it will be destroyed in accordance with our procedures.

The whole documentation relating to the study (study report and its amendments, protocol of study and its amendments and supplements and deviations, raw data, administrative file relating to the study, audit reports, normalized operating procedures) will be archived in a room dedicated for this purpose, for 10 years from the day of sending the study report, except on written request of the customer for different modalities of archiving from those described before. According to our procedures: P-23.After 10 years, all the documentation relating to the study will be destroyed with special care being taken with regard to preserving confidentiality, except on the written request of the customer.

15. STATEMENT

This study performed in the test facility EUROFINS DERMSCAN POLAND Sp. z o.o. Ul. Matuszewskiego 12 80 - 288 GDANSK POLAND, that operates in compliance with the OECD Principles of Good Laboratory Practice, based on Section II of Annex I to the European Parliament and Council Directive 2004/10/EC and embodied in the PL Good Laboratory Practice Regulations 22

The test was performed in accordance with the study plan and study plan supplement. The objectives of this study, as laid down in the study plan, were achieved and nothing occurred to adversely affect the quality or integrity of the study. It was therefore considered that the data generated during the course of this study are valid, and the report provides a true and accurate record of the procedures used and the results obtained.

Regular process-based inspections designed to encompass routine and repetitive procedures at Dermscan Poland Sp z o.o. are conducted monthly.

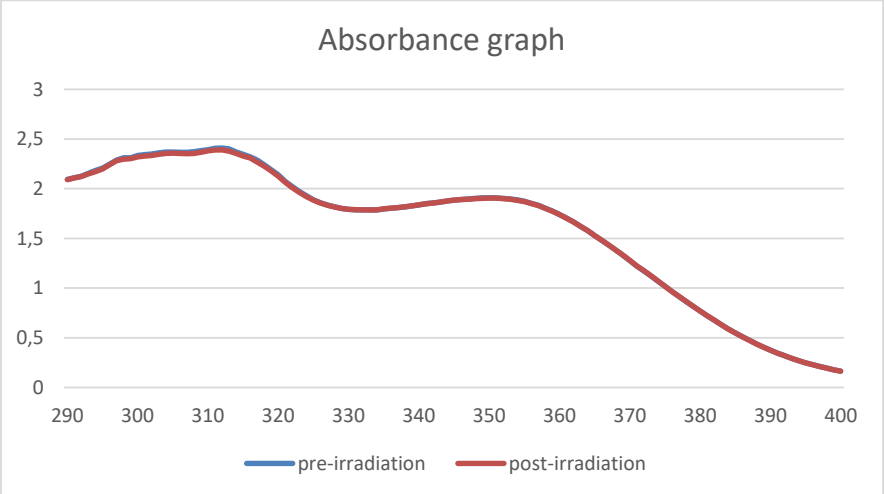
Study Director: **Date: 04.07.2023**

16. APPENDIX

Plate data

Plate	SPF Mean	C Coefficient	UVAPF0	Irradiation Dose (d)	UVAPF
Plate 1	114,48	0,86	12,3	14,76	12,27
Plate 2	181,65	0,78	13,07	15,68	13,19
Plate 3	186,88	0,78	13,32	15,99	13,41
Plate 4	149,79	0,81	12,95	15,53	12,8
Plate 5	0	0	0	0	0
Plate 6	0	0	0	0	0
Plate 7	0	0	0	0	0
Plate 8	0	0	0	0	0
Plate 9	0	0	0	0	0

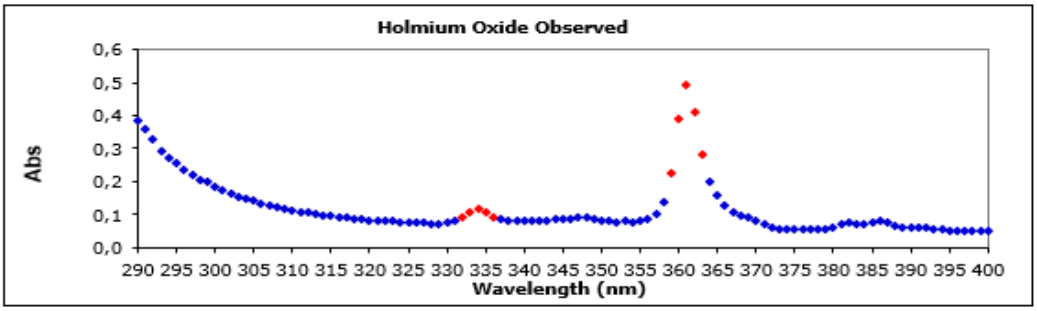
Absorbance graph of tested product



	Solar Simulator	Radiometer	Spectrophotometer
Maufacturer	Solar Light	Solar Light	Labsphere
Model	LS1000-4S-009	PMA2100 with detector PMA2110-UVS	UV2000S UV transmittance analyzer
S/N	27305	27365 with detector 26923	925178650
Date of calibration	15.03.2023	15.03.2023	22.06.2023
Correction Factor	NA	0,84	NA

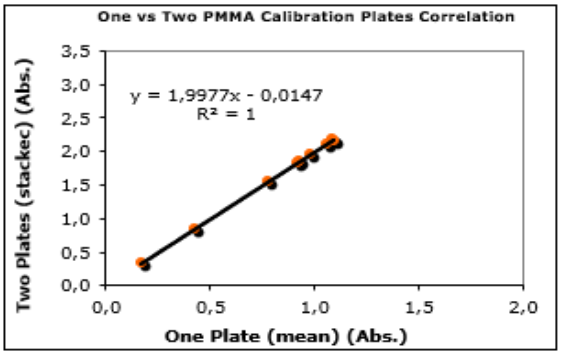
	Peak
Reference wavelength (nm)	361
Measured wavelength (nm)	361
Peak value	0,495
Limit +/-1	0

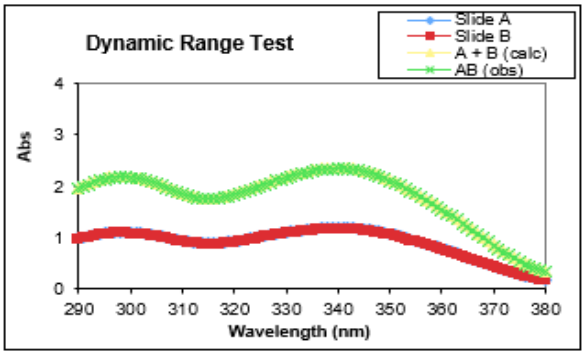
Result
Pass



	Limit	Measurement
Additivity (DO)	2,2	2,34
Linearity (%)	85	99,0
Abs of a plate (340 nm)	1.1 - 1.5	1,18

Result
Pass
Pass
Pass





Spectrophotometer calibration performed: 22.06.2023

HD6 substrate parameters

RESULTS										
Profile		Measured value			Specification					
Parameter*	Unit	Mean	±	SD**	ISO 24443:2021		FDA 2011		Boots Star Rating 2011	
Ra	(µm)	4,615	±	0,076	4,853 ± 0,501	OK	/		/	
Rv	(µm)	12,491	±	0,236	13,042 ± 0,989	OK	/		/	
Rdq	(°)	12,437	±	0,376	11,122 ± 2,032	OK	/		/	
A1	(µm²/mm)	215,163	±	19,989	239,750 ± 70,165	OK	/		/	
Ssc	(1/µm)	0,022	±	0,008	0,033 ± 0,021	OK	/		/	
Vvv	(mm3/mm²)	8,627E-05	±	1,656E-06	1,044,10-4 ± 9,76,10-5	OK	/		/	
Sa	(µm)	5,84	±	0,12	/		2,0 - 7,0	OK	2,0 - 6,0	OK

RESULTS						
Transmission (%T)		Measured value			Specification	
Wavelength	Unit	Mean	±	SD**	ISO 24443:2021	Conclusion
290 nm	%	72,5	±	1,1	> 60%	OK
300 nm	%	78,8	±	1,5	> 69%	OK
320 nm	%	87,7	±	0,2	> 81%	OK

SB6 substrate parameters

RESULTS										
Profile		Measured value			Specification					
Parameter*	Unit	Mean	±	SD**	ISO 24443:2021		FDA 2011		Boots Star Rating 2011	
Ra	(µm)	4,282	±	0,057	4,188 ± 0,514	OK	/		/	
Rv	(µm)	11,458	±	0,239	11,402 ± 2,499	OK	/		/	
Rdq	(°)	10,985	±	0,204	11,004 ± 1,938	OK	/		/	
A1	(µm²/mm)	194,221	±	7,464	238,252 ± 72,663	OK	/		/	
Ssc	(1/µm)	0,029	±	0	0,032 ± 0,015	OK	/		/	
Vvv	(mm3/mm²)	8,167E-04	±	1,620E-05	8,701,10-4 ± 2,325,10-4	OK	/		/	
Sa	(µm)	5,42	±	0,04	/		2,0 - 7,0	OK	2,0 - 6,0	OK

RESULTS						
Transmission (%T)		Measured value			Specification	
Wavelength	Unit	Mean	±	SD**	ISO 24443:2021	Conclusion
290 nm	%	65,0	±	0,1	> 60%	OK
300 nm	%	73,0	±	0,2	> 69%	OK
320 nm	%	82,3	±	0,1	> 81%	OK

**SD: Standard Deviation