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**Report of Evaluation of In Vitro UVA Protection Factor (UVAPF) According to ISO PROTOCOL ISO 24443:2021 – Determination of the Sunscreen UVA protection In Vitro**

**Eurofins Dermatest Sample Number:** UV21p264

**Client:** Millstream Gardens

**Product Description:** MILLSTREAM GARDENS NATURAL SUNSCREEN

**Batch/Formula No.:** 082

**Client PO Number:**

**Test Date:** 31/10/2022

## 1.0 Objective.

The testing was conducted in compliance with the procedures described in ISO 24443:2021. This International Standard describes a method for the determination of sunscreen UVA photoprotection in vitro. The values obtained in the test are then interpretable into a number of regional requirements. For Australia, these are described in AS/NZS 2604:2021. In the EU and ASEAN, similar requirements apply, but labelling expression of values differs.

## 2.0 Sample Description.

A sample labelled MILLSTREAM GARDENS NATURAL SUNSCREEN was received from Millstream Gardens on 12/10/2022 and assigned a Eurofins Dermatest sample number UV21p264.

## 3.0 Test Material Handling.

The record of the sample was entered into a log, identifying the lot number, sample description, batch number, sponsor, date received and tests requested. Samples are retained for a period of two years from the date of receival. All original samples, raw data sheets, technicians notebooks, correspondence files and copies of final reports and remaining specimens are maintained on premises of Eurofins Dermatest in limited access storage files. A duplicate disk copy of final reports is archived separately off site.

## 4.0 Test Principle.

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 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 yields the same measured in vivo SPF value that was determined by in vivo testing. A minimum of 5 in vivo test subject results obtained through testing according to a published ISO method is required for this mean SPF.

In the case of this test study, the SPF value utilised to perform this mathematical adjustment was based on the static result determined through a full study panel according to ISO 24444:2019.

**Note:** Original report released on 1/11/2022 based on an in vivo SPF static result determined through a preliminary 3 subject panel according to ISO 24444:2019 of 27.2. Report was reissued (extrapolation) on 23/11/2022 based on an in vivo SPF static result determined through a full panel study according to ISO 24444:2019 of 28.1.

Samples were exposed to a specific measured dose of UV radiation to account for the photostability characteristics of the test product. The resulting spectral absorbance data provides a useful representation of both the width and height of the UVA protection characteristics of the sunscreen product being tested. The mathematical modelling procedure has been empirically derived to correlate with human in vivo (persistent pigment darkening) test results.

**5.0 Sample Application and Conditioning.**

The sunscreen product was applied to a new untreated roughened PMMA plate (with the roughened side uppermost) by mass, at an application rate of 1.3 mg/cm$^2$ (±1.6 %) for moulded plates and 1.2 mg/cm$^2$ (±1.5 %) for sandblasted plates. For this study moulded PMMA plates were used.

The application dose was determined by measuring the mass loss of the pipette before and after application of the product. Where possible, a positive-displacement automatic pipette was used for this purpose.
Plates were weighed after application phase for any non-volatile product.

The sunscreen was applied as at least twelve small droplets of approximate equal volume, distributed evenly over the whole surface of the plate. The finger was first dipped into the test product and wiped to remove excess product. The product was then applied and immediately spread without the use of a finger cot. Deposit and weighing took no more than 30s.

The spreading technique, as defined in detail on ISO 24443 section 6.4, was followed and time limits were complied with. The PMMA plates with product applied were then held at a temperature of 30˚C ± 2˚C for a period of 30 minutes in order to equilibrate and in similar fashion to the method used in in vitro SPF testing. The same temperature range was used during UV light exposures.

**6.0 Initial Measurements (Pre-Irradiation).**

Each of at least four product-treated plates was placed in the light-path of the spectrophotometer and the absorbance of UV radiation through the sample was determined for each wavelength, from 290nm to 400nm, in 1nm steps. The mean value was determined for each plate.

The initial absorbance curve values are multiplied by a scalar value “C” until the in vitro calculated SPF values are equal to the in vivo measured SPF. In vivo SPF can be used from the mean SPF obtained from screening test (at least 5 valid subjects) or full test (at least 10 valid subjects), measured by a published ISO method.

The initial UVA-PF$_0$ value was calculated, according to the below formula, for the purpose of determining the UVA exposure dose, in a manner similar to the calculation of the initial SPF$_{\text{in vitro}}$.

$$UVA\text{-}PF_0 = \frac{\int_{\lambda=320}^{\lambda=400} P(\lambda)\times I(\lambda)\times d\lambda}{\int_{\lambda=320}^{\lambda=400} P(\lambda)\times I(\lambda)\times 10^{-A_0(\lambda)}\times d\lambda}$$

where :

P(λ) = the PPD action spectrum.
I(λ) = the spectral irradiance from the UVA (320nm – 400nm) source.
$A_0(\lambda)$ = the mean monochromatic absorbance of the product film on the PMMA before UV exposure.
C = the coefficient of adjustment.
d(λ) = the wavelength increment (1nm steps).

**7.0 UV Exposure Dose Calculations**

The UV exposure dose (in J/cm$^2$) for each plate is calculated according to the following formula:

UV Exposure Dose (*D*) = UVA-PF$_0$ x 1,2

The UV Exposure Dose (*D*) limit is 36 J/cm$^2$

**8.0 UV Irradiation of Samples.**

The sample plates were exposed to the radiation from the UV exposure source. During the exposure, the samples were maintained at a temperature of 30˚C +/- 2˚C, the same temperature used for the drying period. The PMMA plates were fixed above a non-reflective UV background to reduce back exposure.

After the UV exposure, the absorbance of the test samples was measured at the same spots as before the UV exposure, using the same spectrophotometer and the UVA-PF is calculated according to the following formula:

$$UVA\text{-}PF = \frac{\int_{\lambda=320}^{\lambda=400} P(\lambda)\times I(\lambda)\times d\lambda}{\int_{\lambda=320}^{\lambda=400} P(\lambda)\times I(\lambda)\times 10^{-A_e(\lambda)\times C}\times d\lambda}$$

where :

$P(\lambda)$ = the PPD action spectrum.
$I(\lambda)$ = the spectral irradiance from the UVA (320nm – 400nm) source.
$A_e(\lambda)$ = the mean monochromatic absorbance of the product film on the PMMA after UV exposure.
C = the coefficient of adjustment.
$d(\lambda)$ = the wavelength increment (1nm steps).

Additional plates were added to the sampling if the 95% confidence interval (CI) was greater than 17% of the mean value of the UVA-PF, until the 95% CI was less than 17 % of the mean UVA-PF value.

**9.0 Calculation of Critical Wavelength.**

The critical wavelength of the product is defined as the wavelength where the area under the absorbance curve between 290nm and 400nm of the irradiated sample represents 90% of the total area and is calculated according to the following formula.

$$\int_{\lambda=290}^{\lambda c} A_e(\lambda)\times d\lambda = 0{,}9\times \int_{\lambda=290}^{\lambda=400} A_e(\lambda)\times d\lambda$$

where:

$Ae(\lambda)$ = the mean absorbance at each wavelength after irradiation.
$d(\lambda)$ = the wavelength increments between measurements.

**10.0 Data Compilation.**

The data was entered into the standardised spreadsheet as provided in ISO 24443:2021. The values obtained from the test were incorporated into this report for the purposes of determining compliance with the various local interpretations, e.g. AS/NZS 2604:2021 and Cosmetics Europe - E.U. Guidelines.

**11.0 Method Validation with Reference Sunscreens.**

The method is controlled through the use of reference sunscreen formulations S2 and P8 in order to verify the test procedure. This validation is performed monthly, together with the calibration of the spectrophotometer used for pre and post irradiation measurements. The UV exposure source is calibrated annually. The UVA-PF test results of the reference sunscreens S2 and P8 must lie between the upper and lower limits set out in the table below. The SPFs for the two formulas were determined from in vivo testing results. SPF 16 is to be used as the in vivo SPF value for S2 for computation purposes and SPF 63.1 for P8.

| Date | Substrate | Reference | Batch No. | | Lower Limit | Upper Limit | Result |
|---|---|---|---|---|---|---|---|
| 6/10/22 | Moulded PMMA | **S2** | **F019** | **UVA-PF** | 10.7 | 14.7 | 13.6 |
| 6/10/22 | Moulded PMMA | **S2** | **F019** | **CW (nm)** | 378 | 382 | 378 |
| **Date** | **Substrate** | **Reference** | **Batch No.** | | **Lower Limit** | **Upper Limit** | **Result** |
| 6/10/22 | Moulded PMMA | **P8** | **F035** | **UVA-PF** | 19.1 | 23.1 | 22.0 |
| 6/10/22 | Moulded PMMA | **P8** | **F035** | **CW (nm)** | 378 | 381 | 380 |

**12.0 Calibration of Irradiation Device.**

The exposure source is calibrated every twelve months or at 2500 hrs lamp running time, according to the requirements of ISO 24443. Total irradiance over the range of 290nm to 400nm must be between 40 and 200 W/m$^2$ and the irradiance ratio of UVA to UVB must lie within the range of 11 to 22. Beam uniformity is required to be at least 90%. All measurements are carried out at 5 locations with the beam.

| **Date** | 27/6/2022 | **Lower Limit** | **Upper Limit** | **Result** |
|---|---|---|---|---|
| **Total UV Irradiance (290 - 400nm)** | | 40 W/m$^2$ | 200 W/m$^2$ | 79 W/m$^2$ |
| **Beam Uniformity** | | >90% | - | 91% |
| **UVA to UVB Irradiance Ratio** | | 11 | 22 | 19 |

**13.0 Calibration of Spectrophotometer.**

**13.1 Wavelength Accuracy:** Using a holmium oxide filter as per instructions in appendix A of ISO 24443:2021 (A.2)

| **Date** | 6/10/22 | **Lower Limit** | **Upper Limit** | **Result** |
|---|---|---|---|---|
| **Wavelength** | | 360nm | 362nm | 361nm |

**13.2 Linearity and Dynamic Range:** Using two transparent UV-stabilised PMMA reference plates, scanned individually and on top of one another as per instructions in appendix A of ISO 24443:2021 (A.3 and A.4)

| **Date** | 6/10/22 | **Limit** | **Result** |
|---|---|---|---|
| **Dynamic Range** | | >2.2 | 2.55 |
| **Linearity** | | $R^2$ >85% | 97.7 |
| **Absorbance of plate at 340nm** | | 1.1 - 1.5 | 1.39 |

**13.3 Plate Transmission Test:** Using a PMMA from the batch used for testing as per instructions in appendix A of ISO 24443:2021 (A.5).

**Substrate:** Moulded PMMA Pate **Manufacturer:** Helioscreen **Batch No.** 521
**Profile Parameters:** Helioscreen Quality Certificate No. 2341 concludes that all roughness parameters are within the ISO 24443:2021 target limits.

| **Date** | 6/10/22 | **Limit** | **Result** |
|---|---|---|---|
| **Wavelength** | | | |
| **290nm** | | >60% | 66.4% |
| **300nm** | | >69% | 72.9% |
| **320nm** | | >81% | 82.3% |

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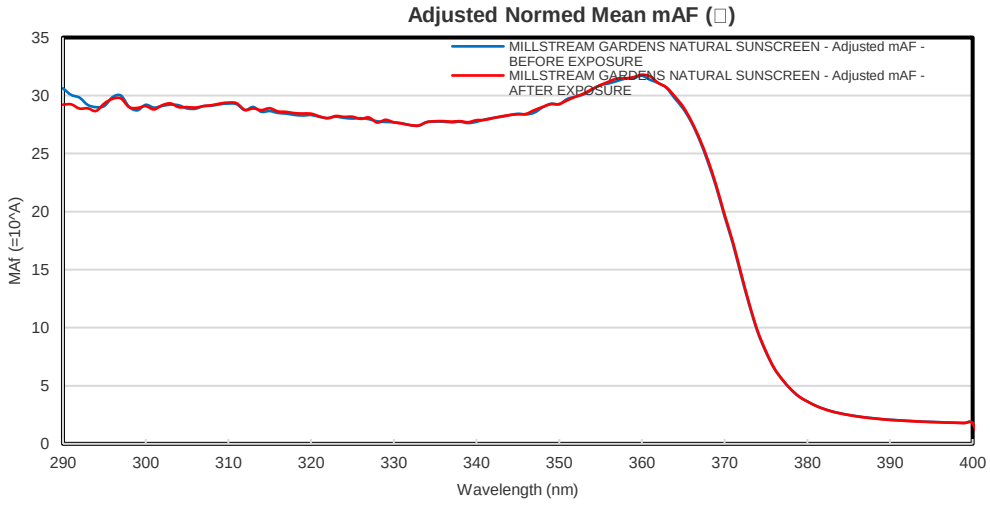
# Experimental Data Summary

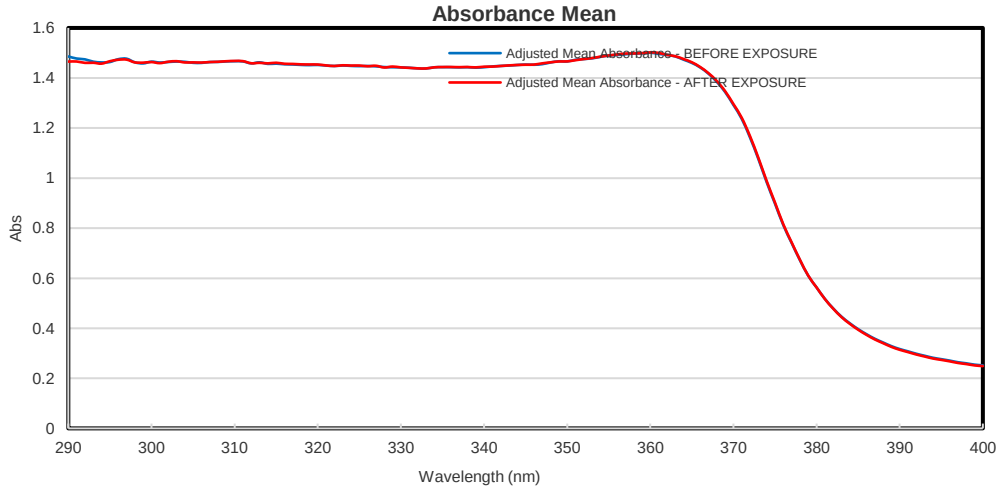
## 14.0 Test Conditions.

**Spectrophotometer:** Labsphere 2000 **UV Exposure Source:** Abet Sun 2000
**In Vivo SPF:** 28.1 (static result determined through a full panel study according to ISO 24444:2019)
**Quantity Applied:** 1.3 mg/cm$^2$ ± 1.6%
**UVA Irradiance:** 79 W/m$^2$
**Plate Drying Temperature:** 30˚C **Plate Drying Time:** 30 mins **Plate Exposure Temperature:** 30˚C
**Radiometer Calibration Factor:** 0.973

## 15.0 Results.

| | Plate 1 | Plate 2 | Plate 3 | Plate 4 | Plate 5 | Plate 6 | Plate 7 | Mean | SD | CI% (<17%) | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Constant "C" (0.6 - 1.6)** | 0.8 | 0.8 | 0.9 | 0.8 | | | | 0.8 | | | |
| **UVA-PF0 (pre-irradiation)** | 14.0 | 13.9 | 13.7 | 13.7 | | | | **13.8** | | | |
| **UVA Exposure Dose (J/cm$^2$)** | 16.5 | 16.3 | 16.1 | 16.1 | | | | 16.3 | | | |
| **UVA Exposure Time (hh:mm:ss)** | 0:35:47 | 0:35:23 | 0:34:58 | 0:35:01 | | | | 0:35:17 | | | |
| **UVA-PF** | 13.9 | 13.7 | 13.8 | 13.8 | | | | **13.8** | 0.1 | 1.1% | [13.7 - 14] |
| **Critical Wavelength (nm)** | 372 | 372 | 372 | 372 | | | | **372** | | | |
| **UVA-PF / Measured SPF** | 0.495 | 0.488 | 0.491 | 0.491 | | | | 0.491 | | | |
| **UVA-PF / Label SPF** | 0.556 | 0.548 | 0.552 | 0.552 | | | | **0.552** | | | |



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# Certificate for Australia and New Zealand - AS/NZS 2604:2021

**Eurofins Dermatest Sample Number:** UV21p264

**Client:** Millstream Gardens

**Product Description:** MILLSTREAM GARDENS NATURAL SUNSCREEN

**Batch/Formula No.:** 082

| UVA-PF Ratio vs Category Description for AS/NZS 2604:2021 | | | | | | Sample Performance | |
|---|---|---|---|---|---|---|---|
| **Tested SPF** | **Labelled SPF** | **Category Description** | **Primary** | **Secondary** | | **UVA-PF Label SPF** | **Pass Fail** |
| | | | | **Skin Care** | **Colour/Lip** | | |
| 1 to 3 | Not allowed | Not allowed | Not allowed | Not allowed | Not allowed | n/a | |
| 4 to 14 | 4 | Low | Compulsory | Compulsory | Optional | 3.450 | Pass |
| | 6 | Low | Compulsory | Compulsory | Optional | 2.300 | Pass |
| | 8 | Low | Compulsory | Compulsory | Optional | 1.725 | Pass |
| | 10 | Low | Compulsory | Compulsory | Optional | 1.380 | Pass |
| 15 to 29 | 15 | Medium or Moderate | Compulsory | Compulsory | Optional | 0.920 | Pass |
| | 20 | | Compulsory | Compulsory | Optional | 0.690 | Pass |
| | 25 | | Compulsory | Compulsory | Optional | 0.552 | Pass |
| 30 to 59 | 30 | High | Compulsory | Compulsory | Compulsory | n/a | n/a |
| | 40 | High | Compulsory | Compulsory | Compulsory | n/a | n/a |
| | 50 | High | Compulsory | Compulsory | Compulsory | n/a | n/a |
| 60 or higher | 50+ | Very High | Compulsory | Compulsory | Compulsory | n/a | n/a |

The sample was evaluated according to the method described in ISO 24443:2021. The in vivo SPF for the pre-irradiation and UVAPF calculations can be used from the mean SPF obtained from a screening study (at least 5 valid subjects) or full test (at least 10 valid subjects), measured by a published ISO method. Extrapolation of UVAPF results from screening is possible if the full test SPF results have a SEM not greater than 3.8 and the variability of the in vivo SPF does not exceed 17%. This sample was evaluated according to the in vivo SPF value of 28.1, which was a **static result determined through a full panel study according to ISO 24444:2019**.

**Results**

- In Vitro UVA-PF: **13.8**
- Critical Wavelength (Post Irradiation) (≥ 370nm): **372**
- Ratio (UVA-PF / Label SPF) (≥ 0.333): **0.552**

According to the AS/NZS 2604:2021 standard the product passed the broad spectrum requirements for a SPF 25 label claim.

**Signed:** _____ **Date:** 23/11/2022

Evgenia Platarou

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# Certificate for E.U. and ASEAN

**Eurofins Dermatest Sample Number:** UV21p264

**Client:** Millstream Gardens

**Product Description:** MILLSTREAM GARDENS NATURAL SUNSCREEN

**Batch/Formula No.:** 082

| UVA-PF Ratio vs Category Descriptions for the European Union and ASEAN | | | | Sample Performance | |
|---|---|---|---|---|---|
| **Tested SPF** | **Labelled SPF** | **Category Description** | **Requirement** | **UVA-PF / Label SPF** | **Pass Fail** |
| | | | | | |
| 1 to 5 | Not allowed | Not allowed | n/a | n/a | |
| 6 to 14 | 6 | Low | Compulsory | 2.300 | Pass |
| | 10 | Low | Compulsory | 1.380 | Pass |
| 15 to 29 | 15 | Medium or Moderate | Compulsory | 0.920 | Pass |
| | 20 | | Compulsory | 0.690 | Pass |
| | 25 | | Compulsory | 0.552 | Pass |
| 30 to 59 | 30 | High | Compulsory | n/a | n/a |
| | 50 | High | Compulsory | n/a | n/a |
| 60 or higher | 50+ | Very High | Compulsory | n/a | n/a |

The sample was evaluated according to the method described in ISO 24443:2021. The in vivo SPF for the pre-irradiation and UVAPF calculations can be used from the mean SPF obtained from a screening study (at least 5 valid subjects) or full test (at least 10 valid subjects), measured by a published ISO method. Extrapolation of UVAPF results from screening is possible if the full test SPF results have a SEM not greater than 3.8 and the variability of the in vivo SPF does not exceed 17%. This sample was evaluated according to the in vivo SPF value of 28.1, which was a **static result determined through a full panel study according to ISO 24444:2019.**

**Results**

- In Vitro UVA-PF: **13.8**
- Critical Wavelength (Post Irradiation) (≥ 370nm): **372**
- Ratio (UVA-PF / Label SPF) (≥ 0.333): **0.552**

According to the European Union Commission the product passed the broad spectrum requirements for a SPF 25 label claim.

**Signed:** _____ **Date:** 23/11/2022

Evgenia Platarou

# Estimation of Other UVA Values

**Eurofins Dermatest Sample Number:** UV21p264

**Client:** Millstream Gardens

**Product Description:** MILLSTREAM GARDENS NATURAL SUNSCREEN

**Batch/Formula No.:** 082

The information on this page is provided only as an indication of possible results. These tests need to be performed separately. This information should not be relied upon for regulatory or marketing purposes.

The estimations are based on an assumed SPF of 28.1.

**JCIA:** Japan, Korea and several other countries require that the UVAPF be performed In vivo.
This estimated PA rating is indicative only.

Based on the post exposure mean UVAPF Value of **13.8**

The Estimated PA Value would be around **PA +++**

| UVAPF Value | UVA Protection Grade |
|---|---|
| Less than 2 | No UVA Protection |
| 2 or more but less than 4 | PA + |
| 4 or more but less than 8 | PA ++ |
| 8 to less than 16 | PA +++ |
| 16 or greater | PA ++++ |

**FDA Broad Spectrum:** - Critical Wavelength (> 370nm) : **370 ± 2nm**

- UVA1/UV (> 0.7) : **0.827**

**BOOTS** The Boots Star rating system is proprietary to Boots Ltd and permission has to be obtained for use of this mark. Indicative values are estimated below. The actual test can be performed by Dermatest if required.

**Indicative Star Rating:** Based on the initial mean UVA:UVB ratio of: **0.76**
and post exposure mean UVA:UVB ratio of: **0.76**

the Boots Star Rating for this product could be expected to be: **3 stars**

| Requirement | | INITIAL mean UVA:UVB Ratio | | | |
|---|---|---|---|---|---|
| | | **0.0 to 0.59** | **0.6 to 0.79** | **0.80 to 0.89** | **0.9 and over** |
| **POST EXPOSURE mean UVA:UVB Ratio** | 0.0 to 0.56 | *No Rating* | *No Rating* | *No Rating* | *No Rating* |
| | 0.57 to 0.75 | *No Rating* | *** | *** | *** |
| | 0.76 to 0.85 | *No Rating* | *** | **** | **** |
| | 0.86 and over | *No Rating* | *** | **** | ***** |