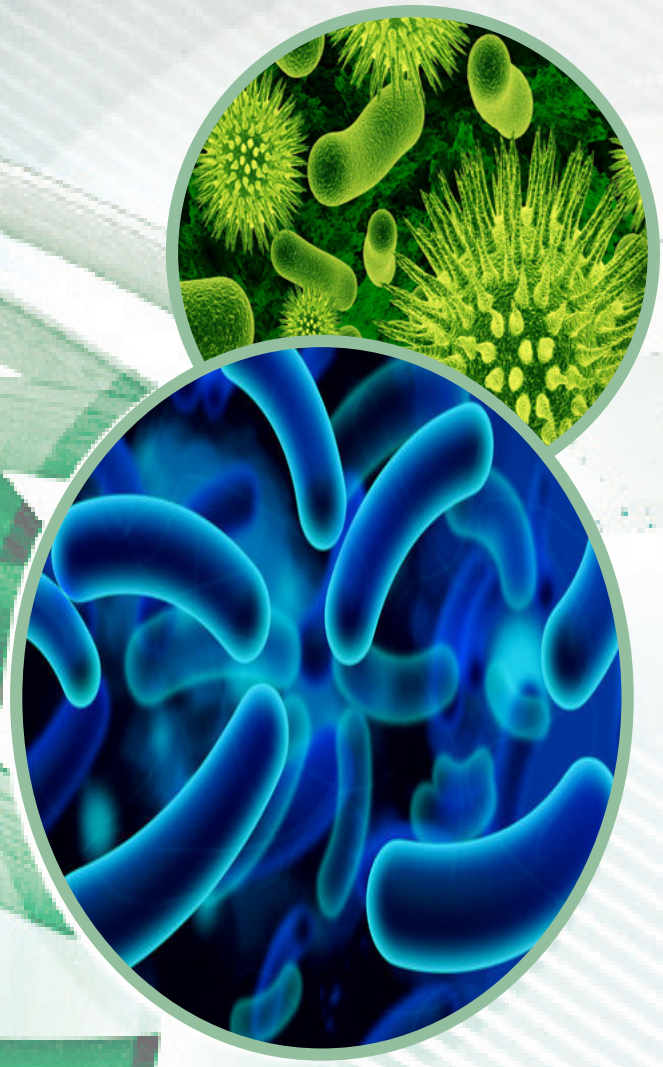
ACTIVE MICRO TECHNOLOGIES

Technical Dossier



ability natural technology Activity
sustainability benefits Ecocert lactobacillus
moisture Cosmos condition peptide
Improving solar choice antimicrobial

Leucidal® Liquid PT

Code Number: M15021

INCI Name: Lactobacillus Ferment

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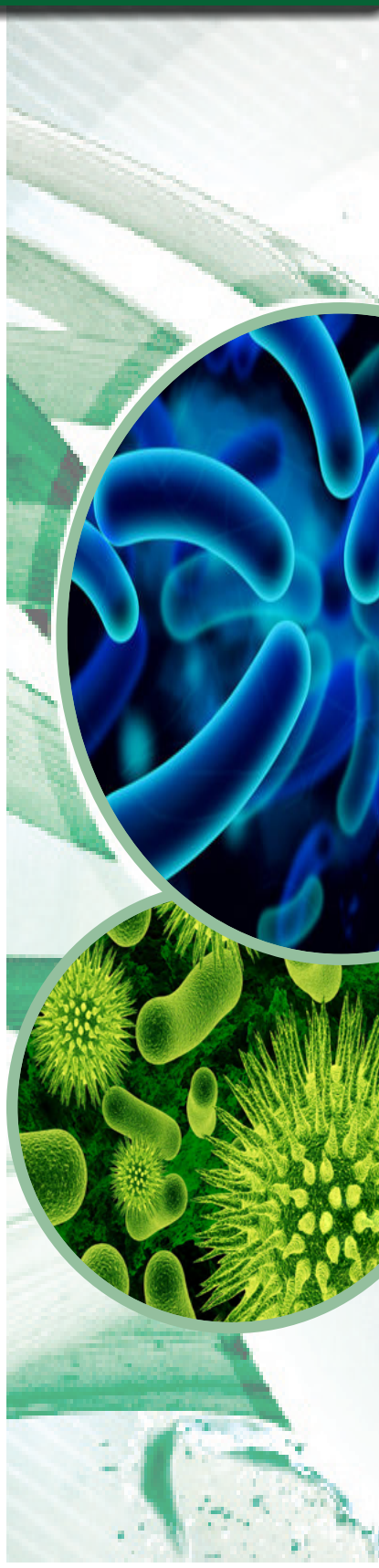
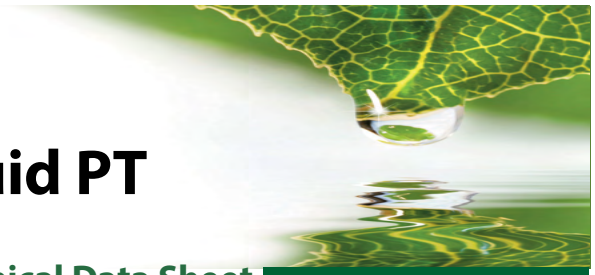


Leucidal® Liquid PT Code Number: M15021
INCI Name: Lactobacillus Ferment



Leucidal® Liquid PT

Technical Data Sheet

BACKGROUND

Consumer choice is the most important factor when it comes to cosmetic sales. Today, a growing number of consumers are opting to move away from synthetic preservatives such as parabens, formaldehyde donors and phenoxyethanol. In addition to public pressure, the use of these synthetic materials in cosmetics is also becoming more strictly regulated. For these reasons, formulators have been actively searching for alternatives to synthetic preservatives that can provide broad spectrum antimicrobial activity.

Active Micro Technologies (AMT) has developed a full line of products derived from naturally occurring compounds that provide broad spectrum antimicrobial protection. As a result, these novel natural antimicrobials can be used as consumer-friendly alternatives to synthetic preservatives in a wide range of cosmetic applications.

Leucidal Liquid PT is a versatile natural antimicrobial, suitable for both emulsions and anhydrous applications such as pressed powders. Pressed powders typically require water-free preservatives capable of providing broad antimicrobial protection. One of the most commonly used preservatives in pressed powders is ethylparaben. Even though it is an effective product, this synthetic preservative is one of the materials that is becoming more strictly regulated, while simultaneously losing consumer preference.

Code Number: M15021
INCI Nomenclature:
Lactobacillus Ferment
INCI Status: Conforms
REACH Status: Fully Compliant
CAS Number: 1686112-36-6
EINECS Number: N/A
Origin: Biotechnology
Lactobacillus
Processing:
GMO Free
No Ethoxylation
No Irradiation
No Sulphonation
No Ethylene Oxide treatment
No Hydrogenation
Additives: None
-Preservatives: None
-Antioxidants: None
Other additives: None
Solvents used: Water
Appearance:
Clear to Slightly Hazy, Pale Yellow to Yellow Liquid
Soluble/Miscible:
Water Soluble
Suggested Use Levels: 2.0%
Suggested Applications:
Skin Conditioner, Antimicrobial

SCIENCE

Leucidal® Liquid PT is a probiotic-based ingredient created by the fermentation of *Lactobacillus* in a defined growth medium and at specific conditions. *Lactobacillus* is one of the species of microorganisms used to produce fermented products such as sauerkraut and kimchi, a Korean dietary staple from cabbage.

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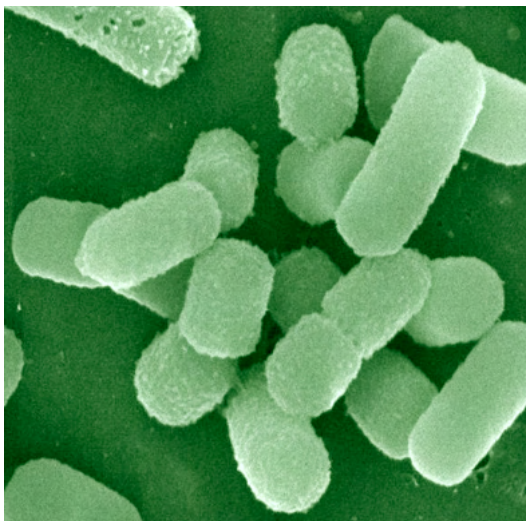
Version 8 - 08.08.16

Leucidal® Liquid PT



Like many members of the lactic acid bacteria family, *Lactobacillus* is capable of restricting the growth of other microorganisms by acidifying its environment. During the fermentation process, in the presence of both standard growth media components and undecylenic acid derived from castor beans, the pH and oxygen levels for *Lactobacillus* are pushed to their limits to induce the production of secondary metabolites as a response to stress. These synergistically active compounds are capable of providing conditioning benefits. An additional growth characteristic of the lactic acid bacteria family is the production of novel antimicrobial peptides. In their natural environment, these antimicrobial peptides provide a competitive advantage to the lactic acid bacteria against other potentially competitive organisms.

After fermentation, lysozyme is added to the culture to facilitate a controlled cell lysis. This step helps ensure the release of the antimicrobial peptides for maximized activity.

BENEFITS

The ability of **Leucidal® Liquid PT** to act as a broad-spectrum antimicrobial is enhanced by the presence of water-soluble undecylenates generated by the *Lactobacillus* during the fermentation process. Undecylenic acid is an oil-soluble, unsaturated fatty acid typically produced by thermal conversion of ricinoleic acid derived from castor oil. Castor oil is a 100% vegetable, biodegradable, natural and renewable resource. In nature, trace quantities of undecylenic acid are also found in human tears and hair.

Biotransformation of undecylenic acid by *Lactobacillus* allows for the generation of water-soluble undecylenates. This process permits the inclusion of this well known anti-fungal agent without the need for undesirable synthetic solvents. In addition to its antimicrobial properties, this odd-carbon number unsaturated fatty acid is a very effective skin moisturizer.

Microorganism	MIC (%)
E. coli	2.00
P. aeruginosa	1.00
S. aureus	0.50
A. brasiliensis	0.50
C. albicans	0.50

Figure 1. MIC Data for **Leucidal® Liquid PT**.

A Minimum Inhibitory Concentration (MIC) test was conducted to evaluate the ability of **Leucidal® Liquid PT** to inhibit the growth of a variety of bacteria and fungi. The results illustrated in Figure 1 show that this material is capable of providing broad spectrum antimicrobial protection.

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Leucidal® Liquid PT

A challenge test using 2.0% **Leucidal® Liquid PT** was also conducted to evaluate the ability of the product to provide antimicrobial protection in cosmetic finished products. An eye shadow formulation was used as the base. The samples were inoculated with *E. coli*, *P. aeruginosa*, *S. aureus*, *C. albicans* and *A. brasiliensis* and incubated for 28 days. During this period, samples were periodically collected and tested for the presence of viable microorganisms.

2.0% Leucidal® Liquid PT in Pressed Powder Challenge Test

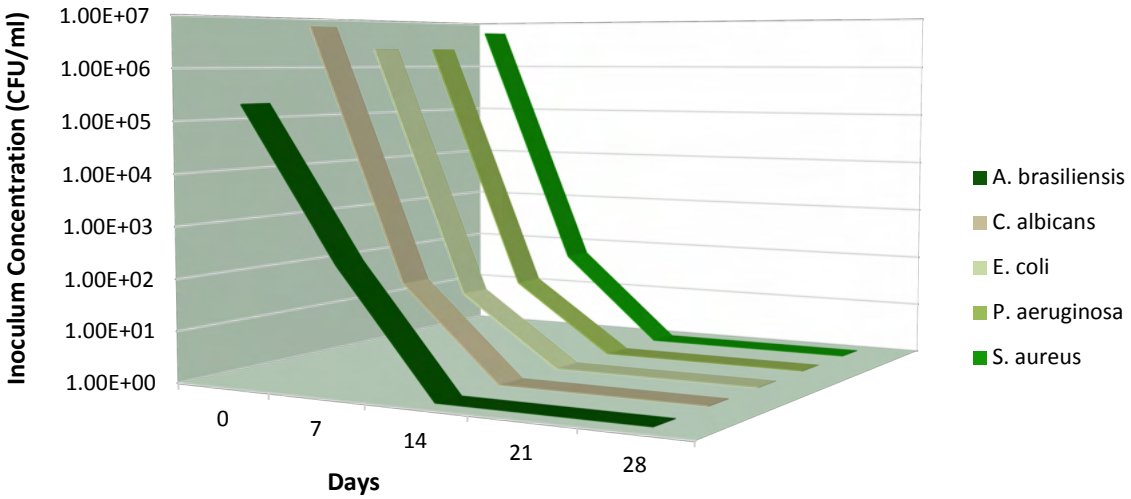

Figure 2. Challenge Test results for Pressed Powder with 2.0% **Leucidal® Liquid PT**. Results show log reduction in viable organisms.

Based on the results in Figure 2, Gram positive and Gram negative bacteria, as well as the yeast and mold were reduced by greater than 99.9% within 7 days of the initial challenge, proving the product's ability to protect cosmetic formulations against microbial contamination.

Eyeshadow using **Leucidal® Liquid PT**	
Sericite PHN	92.00%
Leucidal® Liquid PT	2.00%
Iron Oxide	1.00%
Mineral Oil	5.00%

Figure 3. Formulation for eye shadow used in Challenge Test.

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Leucidal® Liquid PT

A Time Kill Test was performed to determine the change in population of aerobic microorganisms within a specified sampling time when tested against a 2.0% **Leucidal® Liquid PT** solution. The activity of the test material inoculated was evaluated at determine time intervals of 30 seconds, 1, 5, 10 and 30 minutes after the inoculation to determine quantitatively the number of viable microorganisms remaining after the incubation time. As shown in Figure 4, the Gram-positive and Gram-negative bacteria as well as the yeast and mold were reduced by 99.9% within 30 seconds interval of the test after the inoculation.

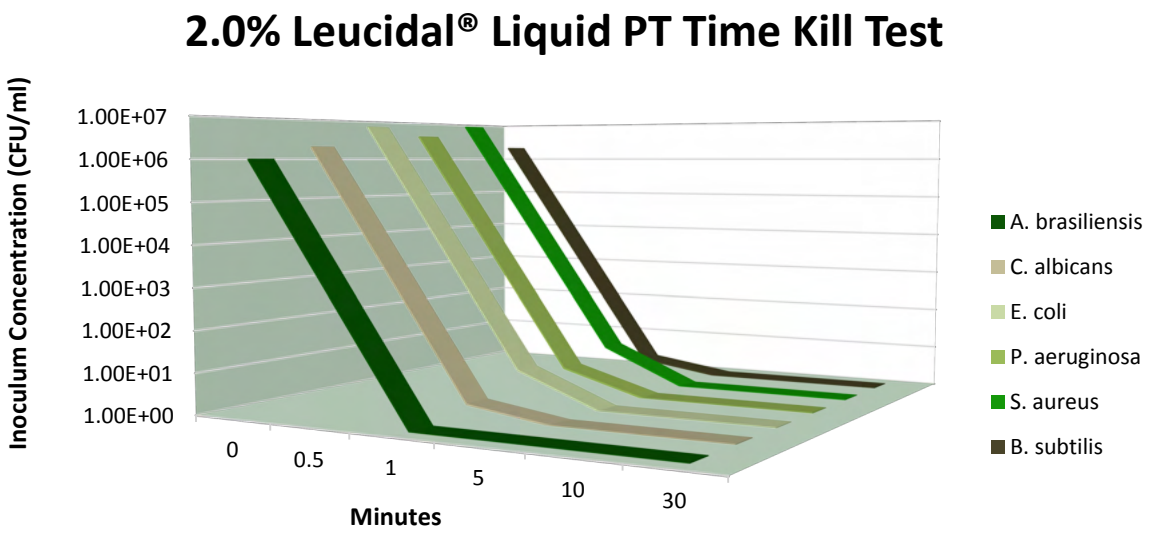

Figure 4. Time Kill Test results for 2.0% **Leucidal® Liquid PT**.

USE RECOMMENDATIONS

We recommend incorporating **Leucidal® Liquid PT** at temperatures lower than 70˚C and a pH between 3 and 8. In addition to this product's effectiveness for powder applications, this product can also be easily incorporated into emulsion systems and water based applications. For color applications, we recommend spraying the product on the pigments along with other ingredients such as binders. Examples of some additional potential applications for **Leucidal® Liquid PT** include hair care products, deodorants and beauty creams.

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Specification

Product Name: Leucidal® Liquid PT
Code Number: M15021
CAS #'s: 1686112-36-6 (or) 9015-54-7
EINECS #'s: N/A (or) 295-635-5
INCI Name: Lactobacillus Ferment

Specification	Parameter
Appearance	Clear to Slightly Hazy Liquid
Color	Pale Yellow to Yellow
Odor	Characteristic
pH (Direct)	7.0 – 8.5
Heavy Metals	< 20 ppm
Lead	< 10 ppm
Arsenic	< 2 ppm
Cadmium	< 1 ppm

DO NOT FREEZE; Store at temperatures between 23 - 28°C;
May sediment upon standing; Mix Well Prior to Use

*Please note: Leucidal® Liquid PT may contain up to 10% water soluble undecylenates.

Product may change appearance if exposed to cold temperatures during shipment or storage. If this happens, please gently warm to 45-50°C and mix until normal appearance is restored.

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Compositional Breakdown

Leucidal® Liquid PT
Code: M15021

Compositional Breakdown:

Ingredient	%
Lactobacillus Ferment	100.00

*** Please note: Leucidal® Liquid PT may contain up to 10% water soluble undecylenates.**

Compositional Breakdown

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Active Micro Technologies hereby confirms that to the best of our knowledge, none of the potential 26 fragrance allergens listed below are present in our finished product or as an intentional component in the raw materials used to manufacture this product. We do not routinely analyze our product for the substances listed below:

ALLERGENS listed in Annex III of EU Cosmetic Regulation(EC) No. 1223/2009	
INCI NAME	CAS Number
Alpha-Isomethyl Ionone	127-51-5
Amyl Cinnamal	122-40-7
Amylcinnamyl Alcohol	101-85-9
Anise Alcohol	105-13-5
Benzyl Alcohol	100-51-6
Benzyl Benzoate	120-51-4
Benzyl Cinnamate	103-41-3
Benzyl Salicylate	118-58-1
Butylphenyl Methylpropional	80-54-6
Cinnamal	104-55-2
Cinnamyl Alcohol	104-54-1
Citral	5392-40-5
Citronellol	106-22-9
Coumarin	91-64-5
Eugenol	97-53-0
Evernia Furfuracea (Treemoss) Extract	90028-67-4
Evernia Prunastri (Oakmoss) Extract	90028-68-5
Farnesol	4602-84-0
Geraniol	106-24-1
Hexyl Cinnamal	101-86-0
Hydroxycitronellal	107-75-5
Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde (Lyral)	31906-04-4
Isoeugenol	97-54-1
Limonene (sum of d, l and dl)	5989-27-5
Linalool	78-70-6
Methyl 2-Octynoate	111-12-6

Compositional Breakdown

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Active Micro Technologies hereby confirms that to the best of our knowledge, none of the pesticides listed below are present in our finished product or as an intentional component in the raw material used to manufacture this product. We do not routinely analyze our product for the substances listed below:

INCI NAME	CAS Number
Alachlor	15972-60-8
Aldrin	309-00-2
Azinphos-methyl	86-50-0
Bromopropylate	18181-80-1
Chlordane (cis and trans)	57-74-9
Chlorfenvinphos	470-90-6
Chlorpyrifos	2921-88-2
Chlorpyrifos-methyl	5598-13-0
Cypermethrin	52315-07-8
DDT	50-29-3
Deltamethrin	52918-63-5
Diazinon	333-41-5
Dichlorvos	62-73-7
Dieldrin	50-57-1
Dithiocarbamates	142-84-7
Endosulfan	115-29-7
Endrin	72-20-8
Ethion	563-12-2
Fenitrothion	122-14-5
Fenvalerate	51630-58-1
Fonofos	944-22-9
Heptachlor	76-44-8
Hexachlorobenzene	118-74-1
Hexachlorocyclohexane	608-73-1
Lindane	58-89-9
Malathion	121-75-5
Methidathion	950-37-8
Parathion	56-38-2
Parathion-methyl	298-00-0
Permethrin	52645-53-1
Phosalone	2310-17-0
Piperonyl butoxide	51-03-6
Pirimiphos-methyl	29232-93-7
Pyrethrins	8003-34-7
Quintozene (sum of 3 items)	82-68-8

Oxygen Radical Absorbance Capacity (ORAC) Assay

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Tradename: Leucidal® Liquid PT

Code: M15021

CAS #: 1686112-36-6 (or) 9015-54-7

Test Request Form #: 249

Lot #: 27794

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Oxygen Radical Absorbance Capacity (ORAC)

Introduction

Reactive oxygen species (ROS) are generated by normal cellular processes, environmental stresses, and UV irradiation. ROS are dangerous to cellular structures and functional molecules (i.e., DNA, proteins, lipids) as they act as strong oxidizing agents or free radicals. The oxygen radical absorbance capacity (ORAC) assay is a standard method used to assess antioxidant capacity of physiological fluids, foods, beverages, and natural products. The assay quantitatively measures a sample's ability to quench free radicals that have the potential to react with and damage cellular components.

Oxygen Radical Absorbance Capacity (ORAC) assay was conducted to assess the antioxidant capacity of **Leucidal® Liquid PT**.

Assay Principle

This assay is based upon the effect of peroxyl radicals generated from the thermal decomposition of 2, 2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) on the signal intensity from the fluorescent probe, fluorescein, in the presence of an oxygen radical absorbing substance. The degree of change is indicative of the amount of radical damage and the presence of antioxidants results in an inhibition in the free radical damage to the fluorescein. The antioxidant protection of the sample can be calculated by comparing it to a set of known standards. Trolox®, a water soluble vitamin E analog, with known antioxidant capabilities is used in this ORAC assay as the standard for measuring the antioxidant capacity of unknown substances. ORAC values, expressed in µM of Trolox® equivalents (TE), are calculated using the area under the curves (AUC) of the test product, Trolox®, and the control materials. Trolox equivalency is used as the benchmark for antioxidant capacity of mixtures since it is difficult to measure individual components.

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Materials

A. Equipment:	Synergy H1 Microplate reader (BioTek Instuments, Winooski, VT); Gen5 software (BioTek Instuments, Winooski, VT); Pipettes
B. Buffers:	75mM Potassium Phosphate (pH 7.4); Deionized H_2O
C. Reagents:	2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) (153mM); 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®); Fluorescein Sodium Salt (4nM)
D. Preparation:	Pre-heat (37˚C) Synergy H1 Microplate reader; Prepare Trolox® standards, sample dilutions, fluorescein solution, and AAPH.
E. Microtitre Plates:	Corning 96 Well Black Side/Clear Bottom Microplates

Methods

Solutions of **Leucidal® Liquid PT** and Trolox® (positive control) were prepared in 75mM potassium phosphate buffer. Materials were prepared at three different concentrations/dilutions. Trolox® was used as a reference for antioxidant capacity and prepared a concentrations ranging from 12.5µM to 200µM in 75mM potassium phosphate buffer.

For the ORAC assay, 25µL of test material and Trolox® were combined with 150µL of fluorescein in 75mM potassium phosphate buffer and incubated in the Synergy HT Microplate reader at 37˚C for 30 minutes. At the end of the incubation period, 25µL of AAPH were pipetted into each well. Fluorescent measurements were then taken every 2 minutes for approximately 2 hours at an excitation wavelength of 485nm and an emissions wavelength of 520nm.

The AUC and Net AUC values of the standards and samples were determined using Gen5 2.0 Data Reduction Software using the below equations:

$$AUC = 0.5 + \frac{R2}{R1} + \frac{R3}{R1} + \frac{R4}{R1} + \cdots + \frac{Rn}{R1} \rightarrow Where\ R\ is\ fluorescence\ reading$$

$$Net\ AUC = AUC_{sample} - AUC_{blank}$$

The standard curve was obtained by plotting the Net AUC of different Trolox® concentrations against their concentration. ORAC values of samples were then calculated automatically using the Gen5 software to interpolate the sample's Net AUC values against the Trolox® standard curve. ORAC measurements for the test material were expressed in micro moles Trolox® equivalents (µMTE), where 1 ORAC unit is equal to 1 µMTE.

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Results

Leucidal® Liquid PT exhibited potent antioxidant activity at a 0.25% concentration.

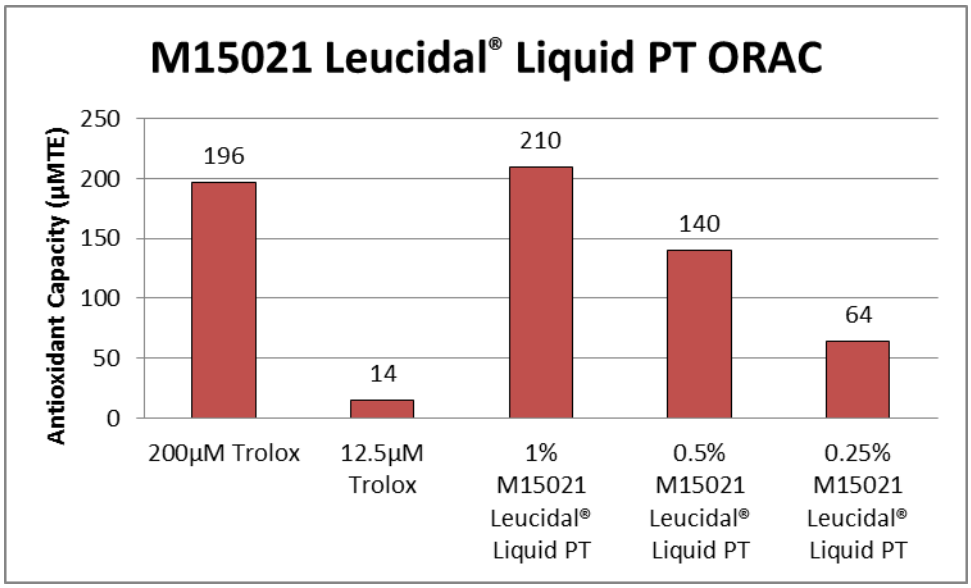

Figure 1: Antioxidant capacities

Discussion

As shown in figure 1, **Leucidal® Liquid PT** exhibited potent antioxidant activity comparable to Trolox®. The antioxidant capacity of **Leucidal® Liquid PT** increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent.

Leucidal® Liquid PT was designed to be moisturizing, conditioning, and provide antimicrobial properties. With the present study we can confirm that this unique ingredient is not only capable of providing functional benefits but it is also capable of providing potent antioxidant benefits when added to cosmetic applications.

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Inhibition Activity Data

Product Name: Leucidal® Liquid PT
Code Number: M15021
Lot Number: 4775P
Test Request Number: 1493
CAS #'s: 1686112-36-6 (or) 9015-54-7
EINECS #'s: N/A (or) 295-635-5
INCI Name: *Lactobacillus* Ferment

Organism (ATCC #)	Minimum Inhibitory Concentration (%)
E.coli #8739	1.0
S. aureus #6538	0.5
P. aeruginosa #9027	2.0
C. albicans #10231	0.5
A. brasiliensis #16404	0.5

QA Signature Monica Beltran

Date 09-08-2015

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Zone of Inhibition Test

Product Name: Leucidal® Liquid PT
Code Number: M15021
Lot Number: 4727P
Test Request Number: 1060
CAS #'s: 1686112-36-6 (or) 9015-54-7
EINECS #'s: N/A (or) 295-635-5
INCI Name: *Lactobacillus* Ferment

Organism (ATCC #)	Zone of Inhibition (mm)
E.coli #8379	8.0
S. aureus #6538	23.6
P. aeruginosa #9027	8.0
C. albicans #10231	22.3
A. brasiliensis #16404	18.3

QA Signature Monica Beltran

Date 02-02-2015

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Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

Leucidal® Liquid PT

Test Request #:

1088

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation pH 3 against bioburden as a function of time.

Study Dates

The study was started on May 6th, 2015 and was completed on July 7th, 2015.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

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Test Method

Fifty grams of Generic Cream Formula pH 3 with 4% Leucidal® Liquid PT was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test formulation.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	1.6×10^6	6.6×10^6	7.7×10^6	4.2×10^5	9.0×10^5
Day 0*	99.950%	>99.999%	>99.999%	99.957%	99.946%
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	99.998%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	3.6×10^6	3.2×10^6	5.6×10^6	4.4×10^5	1.7×10^6
Day 7	>99.999%	>99.999%	>99.999%	99.995%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Cream Formula pH 3 with 4% Leucidal® Liquid PT inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

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Results & Discussion

The results obtained from the Neutralization Test of each product using Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false positive Challenge Test results.

The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream Formula pH 3 with 4% Leucidal® Liquid PT. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

Bacteria – There should be at least a 99.9% (3 log) reduction of vegetative bacteria within 7 days following each challenge and no increase for the duration of the test period.

Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by greater than 99.9% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.999% or greater.

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Phase	Ingredient	Supplier	%
I	Water	-	85.5
	Glycerin	PT. Musim Mas	5.0
	Stearic Acid	Acme Hardesty Oleochemicals	2.5
II	Mineral Oil	RITA	5.0
	Lanolin	RITA	0.5
	Petrolatum	RITA	0.5
	Sepigel 305	Seppic	1.0

Manufacturing Process:

1. Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 80^{0}C while adding the ingredients.

2. Phase II:
In a separate beaker, combine ingredients and heat to 80^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 80^{0}C and mix for 15 minutes. Begin force cooling to 25^{0}C.

3. Check the pH and adjust it if necessary.

Specifications:

Appearance: White to Off-White Emulsion
pH: 5.0 – 8.0
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

The intent of performing an Antimicrobial Efficacy or Challenge test is to evaluate whether an antimicrobial agent or preservation system in a given cosmetic formulation has the ability to prevent the growth of test microorganisms. The test methodology employed by Active Micro Technologies (AMT) is based on the methods published in the CTFA Microbiology Guidelines. AMT's goal is to assist our customers by providing a screening test of a product formulation that is approaching finalization. It is expected that the formulation(s) submitted for Challenge testing contain AMT antimicrobials and have already passed the customer's internal stability tests. It is also anticipated that formal challenge testing of the final formulation will subsequently be performed by the customer at an outside lab of their choosing.

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Challenge Test with
4.0% Leucidal® Liquid PT

Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

Leucidal® Liquid PT

Test Request #:

1089

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation pH 5 against bioburden as a function of time.

Study Dates

The study was started on May 6th, 2015 and was completed on July 7th, 2015.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

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Test Method

Fifty grams of Generic Cream Formula pH 5 with 4% Leucidal® Liquid PT was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test formulation.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	1.6×10^6	6.6×10^6	7.7×10^6	4.2×10^5	9.0×10^5
Day 0*	99.980%	99.999%	99.997%	99.983%	99.954%
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	99.998%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	3.6×10^6	3.2×10^6	5.6×10^6	4.4×10^5	1.7×10^6
Day 7	>99.999%	>99.999%	>99.999%	99.984%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Cream Formula pH 5 with 4% Leucidal® Liquid PT inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

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Results & Discussion

The results obtained from the Neutralization Test of each product using Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false positive Challenge Test results.

The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream Formula pH 5 with 4% Leucidal® Liquid PT. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

Bacteria – There should be at least a 99.9% (3 log) reduction of vegetative bacteria within 7 days following each challenge and no increase for the duration of the test period.

Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by greater than 99.9% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.999% or greater.

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Phase	Ingredient	Supplier	%
I	Water	-	85.5
	Glycerin	PT. Musim Mas	5.0
	Stearic Acid	Acme Hardesty Oleochemicals	2.5
II	Mineral Oil	RITA	5.0
	Lanolin	RITA	0.5
	Petrolatum	RITA	0.5
	Sepigel 305	Seppic	1.0

Manufacturing Process:

1. Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 80^{0}C while adding the ingredients.

2. Phase II:
In a separate beaker, combine ingredients and heat to 80^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 80^{0}C and mix for 15 minutes. Begin force cooling to 25^{0}C.

3. Check the pH and adjust it if necessary.

Specifications:

Appearance: White to Off-White Emulsion
pH: 5.0 – 8.0
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

The intent of performing an Antimicrobial Efficacy or Challenge test is to evaluate whether an antimicrobial agent or preservation system in a given cosmetic formulation has the ability to prevent the growth of test microorganisms. The test methodology employed by Active Micro Technologies (AMT) is based on the methods published in the CTFA Microbiology Guidelines. AMT's goal is to assist our customers by providing a screening test of a product formulation that is approaching finalization. It is expected that the formulation(s) submitted for Challenge testing contain AMT antimicrobials and have already passed the customer's internal stability tests. It is also anticipated that formal challenge testing of the final formulation will subsequently be performed by the customer at an outside lab of their choosing.

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Challenge Test with
2.0% Leucidal® Liquid PT

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(704) 276-7100 • Fax (704) 276-7101

Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

Leucidal® Liquid PT
M15021

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation against bioburden as a function of time.

Study Dates

The study was started on March 21th, 2012 and was completed on May 21th, 2012

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

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Test Method

Fifty grams of Generic Cream pH 5 with 2% Leucidal® Liquid PT was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test solution.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	6.4×10^6	4×10^8	4×10^8	6.6×10^5	1.8×10^7
Day 0*	99.997%	>99.999%	99.999%	99.992%	99.995%
Day 7	>99.999%	>99.999%	99.999%	99.998%	99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	2.5×10^8	2.1×10^6	1.7×10^7	4.6×10^5	8×10^5
Day 7	>99.999%	>99.999%	99.999%	>99.999%	99.999%
Day 14	>99.999%	>99.999%	99.999%	>99.999%	99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	99.999%

Table 1. Challenge Test results for 2% Leucidal® Liquid PT in a Generic Cream pH 5 inoculated on day 0 and re-inoculated on day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

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Results & Discussion

The results obtained from the Neutralization of the product on Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false negative results.

The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream pH 5 with 2% Leucidal® Liquid PT. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

Bacteria – There should be at least a 99.9% (3 log) reduction of vegetative bacteria within 7 days following each challenge and no increase for the duration of the test period.

Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria, as well as the yeast and mold were reduced by greater than 99.9% within 7 days of each challenge. By the end of each 28-day test period these organisms were reduced by more than 99.999%.

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Phase	Ingredient	Supplier	%
I	Water	-	85.5
	Glycerin	PT. Musim Mas	5.0
	Stearic Acid	Acme Hardesty Oleochemicals	2.5
II	Mineral Oil	RITA	5.0
	Lanolin	RITA	0.5
	Petrolatum	RITA	0.5
	Sepigel 305	Seppic	1.0

Manufacturing Process:

1. Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 80^{0}C while adding the ingredients.

2. Phase II:
In a separate beaker, combine ingredients and heat to 80^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 80^{0}C and mix for 15 minutes. Begin force cooling to 25^{0}C.

3. Check the pH and adjust it if necessary.

Specifications:

Appearance: White to Off-White Emulsion
pH: 5.0 – 8.0
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

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Pressed Powder Challenge Test with 2.0% Leucidal® Liquid PT

PCPC Method 6
Determination of Preservation Adequacy of Atypical Personal Care Products

Product

Code: M15021 **Leucidal® Liquid PT**

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a powder formulation against bioburden as a function of time.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Test Method

Twenty grams of a powder formula containing 2% **Leucidal® Liquid PT** was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield 10^4 to 10^6 colony forming units (cfu)/ml. The amount of each inoculum added to each powder sample was no more than 1% of the product weight, as to not alter the products composition.

The inoculated samples were evaluated 0, 2, 7, 14, 21 and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. The table below represents the percent reduction of viable organisms after being introduced into the test powder formulation.

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Results & Discussion

	Organisms				
	E. coli	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
Inoculum (initial)	2.4×10^6	2.4×10^6	5.7×10^6	2.1×10^5	6.5×10^6
Day 0*	>99.999%	>99.999%	>99.999%	99.885%	>99.999%
Day 7	>99.999%	>99.999%	>99.999%	99.971%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

The results of this Challenge Test demonstrate the effectiveness of **Leucidal® Liquid PT** as an antimicrobial agent when used in powder formulations. The recommendations stated in Section 23, Determination of Preservation Adequacy of Atypical Personal Care Products, in the PCPC Microbiology Guidelines are as follows:

Bacteria – There should be at least a 99.9% reduction of vegetative bacteria within 7 days following each challenge and no increase for the duration of the test period.

Yeasts and Molds – There should be at least a 90% reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

Gram positive and Gram negative bacteria, as well as the yeast and mold were reduced by greater than 99.9% within 7 days of each challenge.

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Antimicrobial Efficacy (Challenge) Testing

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Time Kill Test

E2315

Assessment of Antimicrobial Activity Using a Time Kill Procedure

Product

Leucidal® Liquid PT

Test Request #:

1813

Purpose

This study was initiated to measure the change in population of aerobic microorganisms within a specified sampling time when tested against a cosmetic ingredient.

Study Dates

The study was started on March 15th, 2016 and was completed on March 21st, 2016.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Bacillus subtilis* ATCC #6051
5. *Aspergillus brasiliensis:* ATCC #16404
6. *Candida albicans:* ATCC #10231

Neutralization:

Inactivation of the antimicrobial activity of the test material is achieved through the dilution of the test material during the sampling time at specified sampling intervals.

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Test Method

Ten grams of 4% Leucidal® Liquid PT solution was weighed into six individual containers. Each container was inoculated with one of the six test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition. Serial dilutions from each container were performed to enumerate the surviving microorganisms using the Plate Count Technique.

The activity of the test material inoculated was evaluated at determine time intervals of 30 seconds, 1, 5, 10 and 30 minutes after the inoculation to determine quantitatively the number of viable microorganisms remaining after the incubation time.

Organisms	Inoculum Concentration CFU/ml	Percentage of Reduction				
		30 seconds	1 minute	5 minute	10 minute	30 minutes
E.coli * ATCC# 8739	6.7×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
S.aureus ATCC# 6538	8.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
P.aeruginosa ATCC# 9027	4.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
B.subtilis ATCC# 6051	2.1×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
A.brasiliensis ATCC# 16404	1.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
C.albicans ATCC# 10231	2.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%

Table 1. Time Kill Test results for 4% Leucidal® Liquid PT inoculated with 10^6 microorganisms' population. Results show % reduction in viable organisms after inoculation and sampling time intervals.

*Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

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Results & Discussion

The results of this Time Kill Test determine the changes in population of aerobic microorganisms within a specified sampling time when tested against 4% Leucidal® Liquid PT solution.

The Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.9% within 30 seconds interval of the test after the inoculation.

Safety Statement

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Product Name: Leucidal® Liquid PT

Product Code: M15021

INCI Name: Lactobacillus Ferment

INCI Status: Approved

Leucidal® Liquid PT is created by the fermentation of *Lactobacillus* in a defined media under controlled conditions of pH, temperature, and time.

Lactobacillus is a genus of microorganisms used to produce a variety of food products. It is a type of Lactic Acid Bacteria (LAB) and converts various sugars into lactic acid. Any existing LAB in Leucidal® Liquid PT is removed by filtration.

The FDA (Food and Drug Administration) states in sections 201 and 409 of the Federal Food, Drug and Cosmetic Act that "any substance that is intentionally added to food is a food additive, that is subject to review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under conditions of its use or unless the use of the substance is otherwise excluded for the definition of a food additive."[1]

Due to its status as a product of LAB, the Federal Food, Drug and Cosmetic Act classifies materials such as Leucidal® Liquid PT as Generally Recognized as Safe (GRAS). This knowledge combined with the toxicity data provided allows us to support the safety of Leucidal® Liquid PT in cosmetic applications at the recommended use level of 2%.

Due to the restriction placed on the animal testing of cosmetic raw materials, and our internal non-animal testing policy Active Micro Technologies, LLC does not test for NOAEL.

1. Federal Food, Drug and Cosmetic Act. U.S Food and Drug Administration. www.fda.gov.

ACTIVE MICRO TECHNOLOGIES

Dermal and Ocular Irritation Tests

107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

Sample: Leucidal® Liquid PT

Code: M15021

CAS #: 1686112-36-6 (or) 9015-54-7

Test Request Form/Submission #: 1444

Lot #: 30371P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
In Vitro EpiDerm™ Dermal Irritation Test (EPI-200-SIT)
EpiOcular™ Eye Irritation Test (OCL-200-EIT)

SUMMARY

In vitro dermal and ocular irritation studies were conducted to evaluate whether **Leucidal® Liquid PT** would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays.

The product was tested according to the manufacture's protocol. The test article solution was found to be **non-irritating**. Reconstructed human epidermis and cornea epithelial model were incubated in growth media overnight to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substances were applied to the tissue inserts and incubated for 60 minutes for liquid and solid substances in the EpiDerm™ assay and 30 minutes for liquid substances and 90 minutes for solid substances in the EpiOcular™ assay at 37 °C, 5% CO_2, and 95% relative humidity (RH). Tissue inserts were thoroughly washed and transferred to fresh plates with growth media. After post substance dosing incubation is complete, the cell viability test begins. Cell viability is measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2-yl*)], present in the cell mitochondria, into blue formazan salt that is measured after extraction from the tissue. The irritation potential of the test chemical is dictated by the reduction in tissue viability of exposed tissues compared to the negative control.

Under the conditions of this assay, the test article was considered to be **non-irritant**. The negative and positive controls performed as anticipated.

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I. Introduction

A. Purpose

In vitro dermal and ocular irritation studies were conducted to evaluate whether a test article would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays. MatTek Corporation's reconstructed human epidermal and human ocular models are becoming a standard in determining the irritancy potential of test substances. They are able to discriminate between irritants and non-irritants. The EpiDerm™ assay has accuracy for the prediction of UN GHS R38 skin irritating and no-label (non-skin irritating) test substances. The EpiOcular™ assay can differentiate chemicals that have been classified as R36 or R41 from the EU classifications based on Dangerous Substances Directive (DSD) or between the UN GHS Cat 1 and Cat 2 classifications.

II. Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity
B. Equipment:	Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; Pipettes
C. Media/Buffers:	DMEM based medium; DPBS; sterile deionized H_2O
D. Preparation:	Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components
E. Tissue Culture Plates:	Falcon flat bottom 96-well, 24-well, 12-well, and 6-well tissue culture plates
F. Reagents:	MTT (1.0mg/mL); Extraction Solution (Isopropanol); SDS (5%); Methyl Acetate
G. Other:	Nylon Mesh Circles (EPI-MESH); Cotton tip swabs; 1mL tuberculin syringes; Ted Pella micro-spatula; 220mL specimen containers; sterile disposable pipette tips; Parafilm

III. Test Assay

A. Test System

The reconstructed human epidermal model, EpiDerm™, and cornea epithelial model, EpiOcular™, consist of normal human-derived epidermal keratinocytes which have been cultured to form a multilayer, highly differentiated model of the human epidermis and cornea epithelium. These models consist of organized basal, spinous, and granular layers, and the EpiDerm™ systems also contains a multilayer stratum corneum containing intercellular lamellar lipid layers that the EpiOcular™ system is lacking. Both the EpiDerm™ and EpiOcular™ tissues are cultured on specially prepared cell culture inserts.

B. Negative Control

Sterile DPBS and sterile deionized water are used as negative controls for the EpiDerm™ and EpiOcular™ assays, respectfully.

C. Positive Control

Known dermal and eye irritants, 5% SDS solution and Methyl Acetate, were used as positive controls for the EpiDerm™ and EpiOcular™ assays, respectfully.

D. Data Interpretation Procedure

a. EpiDerm™

An irritant is predicted if the mean relative tissue viability of the 3 tissues exposed to the test substance is reduced by 50% of the mean viability of the negative controls and a non-irritant's viability is > 50%.

b. EpiOcular™

An irritant is predicted if the mean relative tissue viability of the 2 tissues exposed to the test substance is reduced by 60% of the mean viability of the negative controls and a non-irritant's viability is > 40%.

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IV. Method

A. Tissue Conditioning

Upon MatTek kit arrival at Active Concepts, LLC the tissue inserts are removed from their shipping medium and transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for 60 minutes. After those 60 minutes the inserts are transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for an additional 18 to 21 hours.

B. Test Substance Exposure

a. EpiDerm™

30µL (liquid) or 25mg (solid) of the undiluted test substance is applied to 3 tissue inserts and allowed to incubate for 60 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

b. EpiOcular™

Each tissue is dosed with 20µL DPBS prior to test substance dosing. 50µL (liquid) or 50mg (solid) of the undiluted test substance is applied to 2 tissue inserts and allowed to incubate for 90 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

C. Tissue Washing and Post Incubation

a. EpiDerm™

All tissue inserts are washed with DPBS, dried with cotton tipped swab, and transferred to fresh media and culture plates. After 24 hours the inserts are again transferred into fresh media and culture plates for an additional 18 to 20 hours.

b. EpiOcular™

Tissue inserts are washed with DPBS and immediately transferred into 5mL of assay medium for 12 to 14 minutes. After this soak the inserts are transferred into fresh media and tissue culture plates for 120 minutes for liquid substances and 18 hours for solid substances.

D. MTT Assay

Tissue inserts are transferred into 300µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% CO_2, and 95% RH. Inserts are then removed from the MTT medium and placed in 2mL of the extraction solution. The plate is sealed and incubated at room temperature in the dark for 24 hours. After extraction is complete the tissue inserts are pierced with forceps and 2 x 200µL aliquots of the blue formazan solution is transferred into a 96 well plate for Optical Density reading. The spectrophotometer reads the 96-well plate using a wavelength of 570 nm.

V. Acceptance Criterion

A. Negative Control

The results of this assay are acceptable if the mean negative control Optical Density (OD_{570}) is ≥ 1.0 and ≤ 2.5 (EpiDerm™) or ≥ 1.0 and ≤ 2.3 (EpiOcular™).

B. Positive Control

a. EpiDerm™

The assay meets the acceptance criterion if the mean viability of positive control tissues expressed as a % of the negative control is ≤ 20%.

b. EpiOcular™

The assay meets the acceptance criterion if the mean viability of positive control tissues is < 60% of control viability.

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C. Standard Deviation
Since each irritancy potential is predicted from the mean viability of 3 tissues for EpiDerm™ and 2 tissues for EpiOcular™, the variability of the replicates should be < 18% for EpiDerm™ and < 20% EpiOcular™.

VI. Results

A. Tissue Characteristics
The tissue inserts included in the MatTek EpiDerm™ and EpiOcular™ assay kits were in good condition, intact, and viable.

B. Tissue Viability Assay
The results are summarized in Figure 1. In no case was the tissue viability ≤ 50% for EpiDerm™ or ≤ 60% for EpiOcular™ in the presence of the test substance. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited substantial loss of tissue viability and cell death.

C. Test Validity
The data obtained from this study met criteria for a valid assay.

VII. Conclusion

Under the conditions of this assay, the test article substance was considered to be **non-irritating**. The negative and positive controls performed as anticipated.

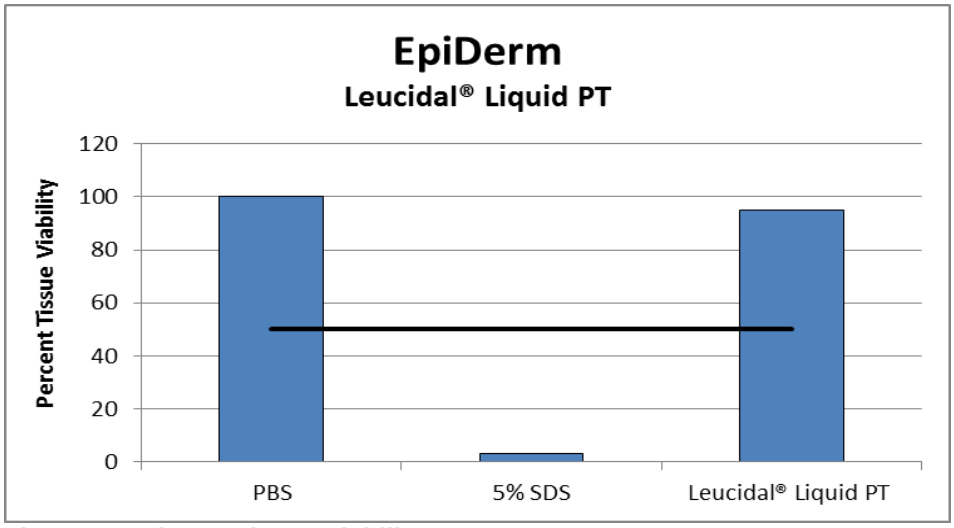

Figure 1: EpiDerm tissue viability

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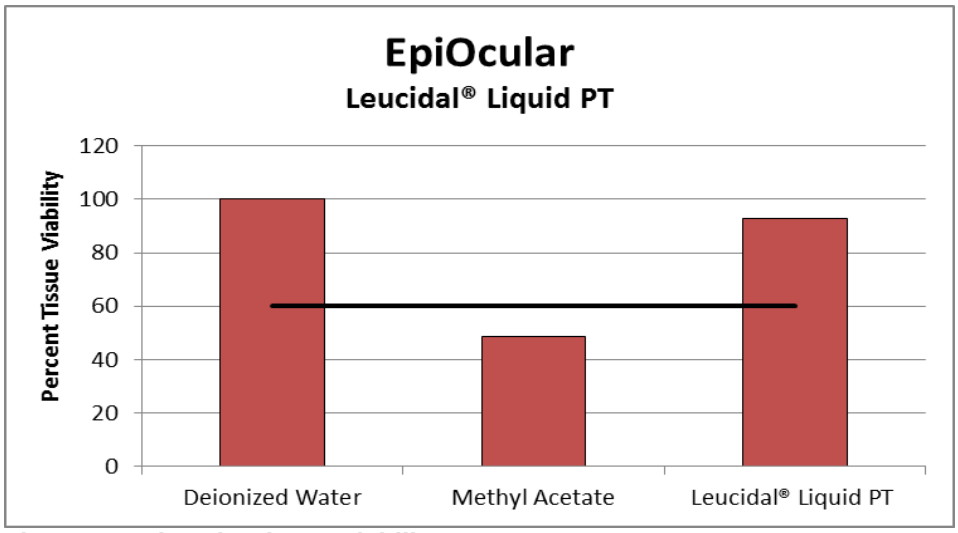

Figure 2: EpiOcular tissue viability

ACTIVE MICRO TECHNOLOGIES

Phototoxicity Assay Analysis

107 Technology Drive • Lincolnton, NC 28092
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Tradename: Leucidal® Liquid PT

Code: M15021

CAS #: 1686112-36-6 (or) 9015-54-7

Test Request Form #: 1136

Lot #: 4727P

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
In Vitro EpiDerm™ Model (EPI-200-SIT) Phototoxicity

SUMMARY

In vitro phototoxicity irritation studies were conducted to evaluate whether **Leucidal® Liquid PT** would induce phototoxic irritation in the EpiDerm™ model assay.

The product was tested according to the manufacturer's protocol. The test article solution was found to be a **non-photoirritant** at concentrations of 0.4%, 1.2%, and 3.7%. Reconstructed human epidermis was incubated in growth media for one hour to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substance was applied to the tissue inserts in five varying concentrations and incubated overnight at 37°C, 5% CO_2, and 95% relative humidity (RH). The following day, the appropriate tissue inserts were irradiated (UVA) for 60 minutes with 1.7 mW/cm^2 (=6 J/cm^2). After substance incubation, irradiation, and washing was completed, the cell viability test was conducted. Cell viability was measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2-yl*)], present in the cell mitochondria, into blue formazan salt that was measured after extraction from the tissue. The photoirritation potential of the test chemical was dictated by the reduction in tissue viability of UVA exposed tissues compared to non-UVA exposed tissues.

Under the conditions of this assay, the test article was considered to be **non-phototoxic** at concentrations of 0.4%, 1.2%, and 3.7%. The negative and positive controls performed as anticipated.

I. Introduction

A. Purpose

In vitro dermal phototoxicity study was conducted to evaluate whether a test article would induce photoirritation in the EpiDerm™ model assay. MatTek Corporation's reconstructed human epidermal model is becoming a standard in determining the phototoxicity potential of a test substance. This assay is able to discriminate between photoirritants and non-photoirritants at varying concentrations.

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II. Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity
B. Equipment:	Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; UVA-vis Irradiation Equipment; UVA meter; Pipettes
C. Media/Buffers:	Dulbecco's Modified Eagle Medium (DMEM) based medium; Dulbecco's Phosphate-Buffered Saline (DPBS); sterile deionized H_2O
D. Preparation:	Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components
E. Tissue Culture Plates:	Falcon flat bottom 96-well, 24-well, and 6-well tissue culture plates
F. Reagents:	MTT (*3-4,5-dimethyl thiazole 2-yl*) (1.0mg/mL); Extraction Solution (Isopropanol); Chlorpromazine; Triton X-100 (1%)
G. Other:	Wash bottle; sterile disposable pipette tips; Parafilm; forceps

III. Test Assay

A. Test System

The reconstructed human epidermal model, EpiDerm™ consists of normal human-derived epidermal keratinocytes which have been cultured to form a multilayer, highly differentiated model of the human epidermis. This model consists of organized basal, spinous, and granular layers, and contains a multilayer stratum corneum containing intercellular lamellar lipid layers. The EpiDerm™ tissues are cultured on specially prepared cell culture inserts.

B. Negative Control

Sterile deionized water is used as the negative controls for the EpiDerm™ Phototoxicity assay.

C. Positive Control

Concentrations of chloropromazine, ranging from 0.001% to 0.1%, were used as positive controls for the EpiDerm™ Phototoxicity assay.

D. Data Interpretation Procedure

A photoirritant is predicted if the mean relative tissue viability of the 2 tissues exposed to the test substance and 60 minutes of 6 J/cm^2 is reduced by 20% compared to the non-irradiated control tissues.

IV. Method

A. Tissue Conditioning

Upon MatTek kit arrival at Active Micro Technologies, LLC the tissue inserts are removed from their shipping medium and transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for 60 minutes. After those 60 minutes the inserts are transferred into fresh media and tissue culture plates and tissue insert dosing begins.

B. Test Substance Exposure

50µL of the diluted test substance in their respective concentrations are applied to 2 tissue inserts and allowed to incubate for overnight in a humidified incubator (37°C, 5% CO_2, 95% RH).

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C. Tissue Irradiation

Tissue inserts in their 6-well plates are UVA-irradiated for 60 minutes with 6 J/cm^2 at room temperature. The non-irradiated tissue inserts are incubated at room temperature in the dark.

D. Tissue Washing and Post Incubation

After UVA-irradiation and dark incubation is complete the tissue inserts are washed using sterile DPBS and transferred to fresh 6-well plates and media for overnight incubation at 37°C, 5% CO_2, 95% RH.

E. MTT Assay

Tissue inserts are transferred into 300µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% CO_2, and 95% RH. Inserts are then removed from the MTT medium and placed in 2mL of the extraction solution. The plate is sealed and incubated at room temperature in the dark for 24 hours. After extraction is complete the tissue inserts are pierced with forceps and 2 x 200µL aliquots of the blue formazan solution is transferred into a 96 well plate for Optical Density reading. The spectrophotometer reads the 96-well plate using a wavelength of 570 nm.

V. Acceptance Criterion

A. Negative Control

The results of this assay are acceptable if the mean negative control Optical Density (OD_{570}) is ≥ 0.8.

B. Positive Control

The assay meets the acceptance criterion if a dose dependent reduction in cell viability in the UVA-irradiated tissues is between 0.00316% and 0.0316%.

C. Standard Deviation

Since the phototoxicity potential is predicted from the mean viability of 2 tissues for the EpiDerm™ Phototoxicity Protocol, the variability of the replicates should not exceed 30%.

VI. Results

A. Tissue Characteristics

The tissue inserts included in the MatTek EpiDerm™ assay kit were in good condition, intact, and viable.

B. Tissue Viability Assay

The results are summarized in Figure 1. Cell viability is calculated for each tissue as a percentage of the corresponding vehicle control either irradiated or non-irradiated. Tissue viability was not reduced by 20% in the presence of the test substance and UVA-irradiation at concentrations of 0.4%, 1.23%, and 3.7%. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited dose dependent loss of tissue viability and cell death.

C. Test Validity

The data obtained from this study met criteria for a valid assay. The negative and positive controls performed as anticipated.

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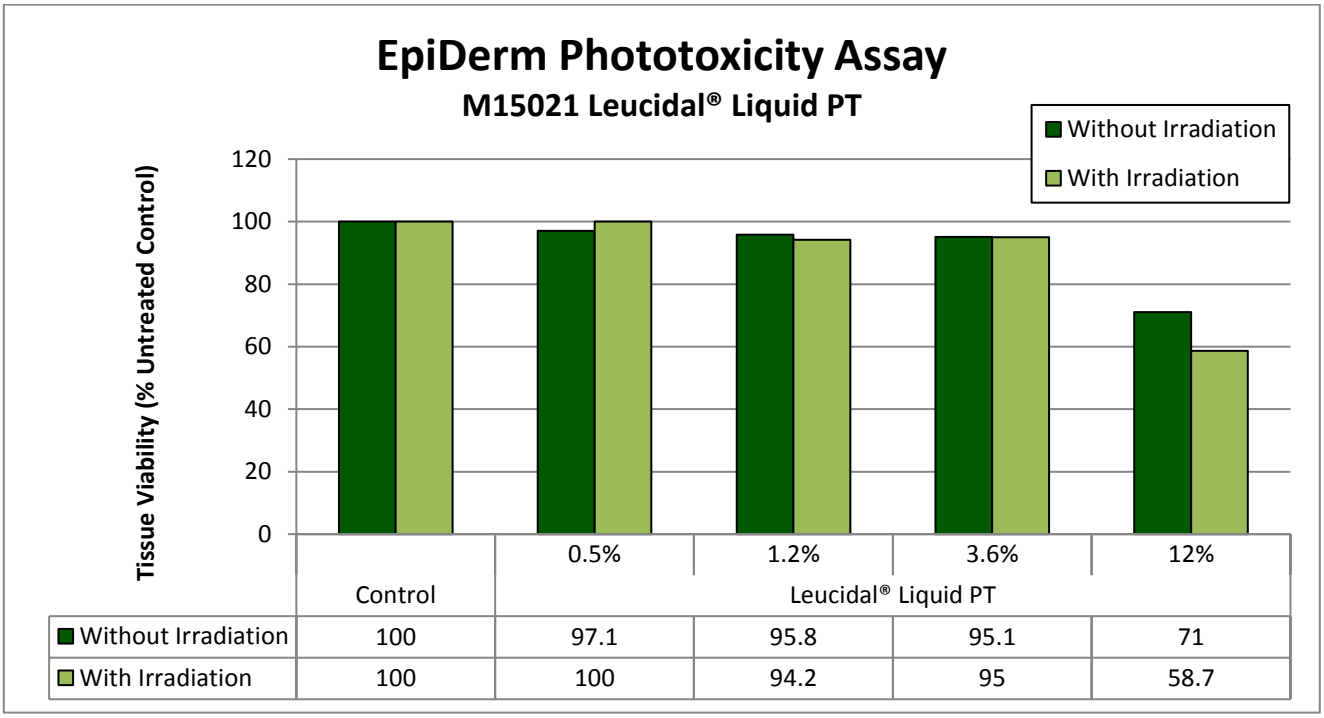
VII. Conclusion

Phototoxicity (photoirritation) is defined as an acute toxic response that is elicited after exposure of the skin to certain chemicals and subsequent exposure to light. Under the conditions of this assay, the test article substance was considered to be **non-phototoxic** at concentrations of 0.4%, 1.2%, and 3.7%. There is a decrease in viability at the 11% test concentration with and without irradiation. Using any test substance at this high of a concentration will have noticeable effects on cellular viability due to the fact that that substance is replacing the cell's nutrients. We can safely say that **Leucidal® Liquid PT** is not a photoirritant when used at the suggested use levels of up to 2%.



	Control	0.5%	1.2%	3.6%	12%
Without Irradiation	100	97.1	95.8	95.1	71
With Irradiation	100	100	94.2	95	58.7

Figure 1: EpiDerm Phototoxicity Graph

OECD 201 Freshwater Alga Growth Inhibition Test

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Tradename: Leucidal® Liquid PT

Code: M15021

CAS #: 1686112-36-6 (or) 9015-54-7

Test Request Form #: 9385

Lot #: 804600

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
OECD 201
Freshwater Alga Growth Inhibition Test

Introduction

The purpose of the present study is to determine the toxicity of **Leucidal® Liquid PT** by exposing the exponentially growing test organism *Pseudokirchneriella subcapitata* to the test substance for 72 hours and measuring the growth and growth inhibition through cell counting against the control. The response is evaluated as a function of the exposure concentration in comparison with the average growth of replicate, unexposed control cultures.

OECD Guideline 201 on "Fresh Alga and Cyanobacteria, Growth Inhibition Test", adopted in 1984, extended the guideline to include additional species and update it to meet the requirements for hazard assessment and classification of chemicals in 2006.

Assay Principle

Pseudokirchneriella subcapitata, are exposed to the test substance at a range of concentrations for a period of 72 hours. The cultures are allowed unrestricted exponential growth under nutrient sufficient conditions and continuous light for a sufficient period of time to measure reduction of the specific growth rate. Growth and growth inhibition are quantified from measurements of the algal biomass as a function of time. The test endpoint is inhibition of growth, expressed as the logarithmic increase in biomass during the 72 hour exposure period. The results are analyzed in order to calculate the EC_{10} and EC_{20} at 72 hours. The response is evaluated as a function of the exposure concentration in comparison with the average growth of replicate, unexposed control cultures.

A reliable analytical method for the quantification of the substance in the test solutions with reported recovery efficiency and limit of determination should be available. A reference substance may be tested for EC_{50} as a means of assuring that the test conditions are reliable.

Analysis of the concentration of the test substance at the start and end of the test of a low and high test concentration around the expected EC_{50} may be sufficient where it is likely that exposures concentrations will vary less than 20% from the nominal values during the test.

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Materials

- Glass Flasks with air-permeable stopper
- Automated Pipette
- pH Meter
- Temperature Control Apparatus
- Microscope with counting chamber
- *Pseudokirchneriella subcapitata* (ATCC 22662)
- Gorham's medium for algae (ATCC MD-0625)

Methods

Test Conditions

- Incoculum Culture
  - Inoculum culture is incubated under the same conditions as the test cultures for 2-4 days allowing for exponential growth to prevail before the start of the test. This is done to ensure that growth is within the normal range for the test strain under the culturing conditions.
- Initial Biomass
  - The initial biomass in the test cultures must be the same in all test cultures and sufficiently low to allow exponential growth throughout the incubation period without risk of nutrient depletion. The initial biomass should not exceed 0.5 mg/L as dry weight.
- Exposure Period
  - 72 hours
- Number of Test Organisms
  - *Pseudokirchneriella subcapitata* 5 x 10^{3-4} cells/ml
- Test Concentration
  - Adopt a concentration range of at least 5 concentrations, causing a range of 5-75% inhibition of algal growth rate expressed as E_rC_x
- Culture Method
  - Illumination: Continuous uniform fluorescent illumination
  - Temperature: The temperature is between 21°C to 24°C
  - pH: pH of the control medium should not increase be more than 1.5 units during test

Measurement of Test Substance Concentrations

- Measurement of biomass is done by manual cell counting by microscope.
- Algal biomass in each flask is determined daily during test period.
- At the beginning and end of exposure, measure test substance concentrations at the lowest and highest test concentration groups.
  - For volatile or adsorptive substances, additional measurements are recommended at 24 hours intervals during exposure period.

Observation

- Microscopic observation should be performed to verify a normal and healthy appearance of the inoculum culture and to observe any abnormal appearance of the algae at the end of the test.

Test Condition Measurements

- Measure pH in the control and at the highest test concentration at the beginning and end of the exposure period.
- Water temperature should be measured at the beginning and end of the exposure period.

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Data and Reporting

I. Data
   a. Tabulate the estimated biomass concentration in test cultures and controls together with the concentrations of test materials and the times of measurement, recorded with a resolution of at least whole hours, to produce plots of growth curves.
   b. For each response variable to be analyzed, use the concentration-response relationship to calculate point estimates of EC_x values. Recent scientific developments have led to a recommendation of abandoning the concept of NOEC and replacing it with regression based point estimates EC_x, specifically EC_{10} and EC_{20}.

II. Test Report
   a. The test report must include the following:
      i. Test substance:
         1. Physical nature and relevant physical-chemical properties
         2. Chemical identification data, including purity
      ii. Test species:
         1. Source and species of *Pseudokirchneriella subcapitata*, supplier of source (if known), and the culture conditions (including source, kind and amount of food, feeding frequency)
      iii. Test conditions:
         1. Description of test vessels: type and volume of vessels, volume of solution, density of *Pseudokirchneriella subcapitata* per test vessel, number of test vessels (replicates) per concentration
         2. Methods of preparation of stock and test solutions including the use of any solvent or dispersants, concentrations used
         3. Details of dilution water: source and water quality characteristics (pH, hardness, Ca/Mg ratio, Na/K ratio, alkalinity, conductivity, etc); composition of reconstituted water if used
         4. Incubation conditions: temperature, light intensity and periodicity, pH, etc.
      iv. Results:
         1. The nominal test concentrations and the result of all analyses to determine the concentration of the test substance in the test vessels; the recovery efficiency of the method and the limit of determination should also be reported
         2. All physical-chemical measurements of temperature and pH made during the test
         3. The EC_{10} and EC_{20} at 72 hours for percent inhibition with confidence intervals and graphs of the fitted model used for calculation, the slopes of the dose-response curves and their standard error; statistical procedures used for determination of EC_{10} and EC_{20}.

$$Percent\ (\%)Inhibition = \frac{\mu c - \mu T}{\mu c} \times 100$$

μ_c: mean value for average specific growth rate (μ) in the control group
μ_T: average specific growth rate for the treatment replicate

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Results

General Information:

Name of new chemical substance	Leucidal® Liquid PT		
INCI Nomenclature	Lactobacillus Ferment		
CAS number	1686112-36-6 (or) 9015-54-7		
Formulation Method	Fermentation		
Molecular weight	4890 Da		
Purity of the new chemical substance used for the test (%)	100%		
Lot number of the new chemical substance used for the test	804600		
Names and contents of impurities	n/a		
Solubility in water	Soluble		
Properties at room temperature	Clear to Slightly Hazy Pale Yellow to Yellow Liquid, Characteristic Odor		
Stability	Stable Under Normal Conditions		
Solubility in solvents, etc.	**Solvent**	**Solubility**	**Stability in solvent**
	N/A	N/A	N/A

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Test Materials and Methods:

Items			**Contents**
Test Organisms	Species		*Pseudokirchneriella subcapitata*
	Source		ATCC
	Reference substance (EC_{50})		3,5-dichlorophenol
Culture	Kind of Medium		Gorham's Medium for Algae
	Conditions (Temperature)		22°C ± 2°C
Test Conditions	Test Vessel		Glass
	Material Water	Kind	Deionized
		Hardness	250 mg/L
		pH	7.4
	Date of Exposure		04/11/2022
	Test Concentrations		200, 89.4, 42.3, 19.2, 7.8 mg/L
	Number of organisms		5 x 103-4 cells/ml
	Number of Replicates	Exposure Group	4
		Control Group	4
	Test Solution Volume		5 mL
	Vehicle	Use or Not	N/A
		Kind	N/A
		Concentration	N/A
		Number of Replicates	N/A
	Photoperiod		Continuous

Test Results:

Items		**Contents**
Toxicity Value	Percent Inhibition EC_{10} and EC_{20}	138.12 mg/L and 240.99 mg/L
Exposure Concentrations Used for Calculation	Nominal Values	200, 89.4, 42.3, 19.2, 7.8 mg/L
Remarks		Not harmful to aquatic organisms

Discussion

After 72 hours, the percent inhibition for **Leucidal® Liquid PT** was determined to be 138.12 mg/L EC_{10} and 240.99 mg/L EC_{20}. The conditions of OECD guideline 201 for the validity of the test were adhered to, this product is not classified and therefore not harmful to aquatic organisms.

OECD 301B Ready Biodegradability Assay

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Tradename: Leucidal® Liquid PT

Code: M15021

CAS #: 1686112-36-6 (or) 9015-54-7

Test Request Form #: 1046

Lot #: 4727P

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
OECD 301 B
Ready Biodegradability: CO_2 Evolution (Modified Sturm Test)

Introduction

A study was conducted to assess the ready biodegradability of **Leucidal® Liquid PT** in an aerobic aqueous medium. In the OECD guideline 301 for ready biodegradability, six methods are provided as options. This report uses method B, CO_2 Evolution, also known as a Modified Sturm Test. This method was chosen based on the solubility, volatility, and adsorbing capabilities of the test sample.

Assay Principle

A solution or suspension of the test substance in a mineral medium is inoculated and incubated under aerobic conditions in the dark or in diffuse light. The amount of DOC (Dissolved Organic Carbon) in the test solution due to the inoculum should be kept as low as possible compared to the amount of organic carbon due to the test substance. Allowance is made for the endogenous activity of the inoculum by running parallel blanks with inoculum but without test substance. A reference compound is run in parallel to check the procedures' operation.

In general, degradation is followed by the determination of parameters such as DOC, carbon dioxide production, and oxygen uptake. Measurements are taken at sufficiently frequent intervals to allow the identification of the beginning and end of biodegradation.

Normally this test lasts for 28 days, but it may be ended before that time if the biodegradation curve reaches a plateau for at least three determinations. Tests may also be prolonged beyond 28 days when the curve shows that biodegradation has started but the plateau has not yet been reached. In such cases the test substance would not be classified as readily biodegradable.

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The pass levels for ready biodegradability are 70% removal of DOC and 60% of ThOD (Theoretical Oxygen Demand) or $ThCO_2$ (Theoretical Carbon Dioxide) production for respirometric methods. They are lower in the respirometric methods since, as some of the carbon from the test chemical is incorporated into new cells, the percentage of CO_2 produced is lower than the percentage of carbon being used. These pass values have to be reached in a 10-day window within the 28-day period of the test. The 10-day window begins when the degree of biodegradation has reached 10% DOC, ThOD, or $ThCO_2$ and must end before day 28 of the test. Test substances which reach the pass levels after the 28-day period are not deemed to be readily biodegradable.

In order to check the procedure, reference compounds which meet the criteria for ready biodegradability are tested by setting up an appropriate vessel in parallel as part of normal test runs. Suitable compounds are freshly distilled aniline, sodium acetate, and sodium benzoate. These compounds all degrade in this method even when no inoculum is deliberately added.

Because of the nature of biodegradation and of the mixed bacterial populations used as inocula, determinations should be carried out at least in duplicate. It is usually found that the larger the concentration of microorganisms initially added to the test medium, the smaller the variation between replicates.

Materials

- Water
  - Deionized or distilled, free from inhibitory concentrations of toxic substances
  - Must contain no more than 10% of the organic carbon content introduced by the test material
  - Use only one batch of water for each series of tests
- Mineral media
  - To prepare the mineral medium, mix 10 mL of solution A with 800 mL water. Then add 1 mL each of solutions B, C, and D and make up to 1 liter with water.
  - Solution A (Dissolve in water and make up to 1 liter; pH 7.4)
    - Potassium dihydrogen orthophosphate, KH_2PO.....8.5g
    - Dipotassium hydrogen orthophosphate, K_2HPO_4.....21.8g
    - Disodium hydrogen orthophosphate dehydrate, $Na_2HPO_4.2H_2O$.....33.4g
    - Ammonium chloride, NH_4Cl.....0.5g
  - Solution B (Dissolve in water and make up to 1 liter)
    - Calcium chloride, anhydrous, $CaCl_2$.....27.50g
      Or
    - Calcium chloride dehydrate, $CaCl_2.2H_2O$.....36.40g
  - Solution C (Dissolve in water and make up to 1 liter)
    - Magnesium sulphate heptahydrate, $MgSO_4.7H_2O$..... 22.50g
  - Solution D (Dissolve in water and make up to 1 liter.)
    - Iron (III) chloride hexahydrate, $FeCl_3.6H_2O$.....0.25g
  - Flasks, 2-5 liters each, fitted with aeration tubes reaching nearly to the bottoms of the vessels and an outlet
  - Magnetic stirrers
  - Gas absorption bottles
  - Device for controlling and measuring air flow
  - Apparatus for carbon dioxide scrubbing, for preparation of air which is free from carbon dioxide; alternatively, a mixture of CO_2-free oxygen and CO_2-free nitrogen from gas cylinders in the correct proportions (20% O_2 : 80% N_2)
  - Device for determination of carbon dioxide, either titrimetrically or by some form of inorganic carbon analyzer

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- Stock solutions of test substances
  - When solubility of the substance exceeds 1 g/L, dissolve 1-10 g, as appropriate, of test or reference substance in water and make up to 1 liter. Otherwise, prepare stock solutions in mineral medium or add the chemical directly to the mineral medium.
- Inoculum
  - The inoculum may be derived from the following sources
    - Activated sludge
    - Sewage effluents
    - Surface waters
    - Soils
    - Or from a mixture of these.
  - Inoculum may be pre-conditioned to the experimental conditions, but not pre-adapted to the test substance. Pre-conditioning consists of aerating activated sludge in mineral medium or secondary effluent for 5-7 days at the test temperature. Pre-conditioning sometimes improves the precision of the test method by reducing blank values.

Methods

I. Preparation of flasks: As an example, the following volumes and weights indicate the values for 5-liter flasks containing 3 liters of suspension. If smaller volumes are used, modify the values accordingly.
   a. To each 5-liter flask, add 2,400 mL mineral medium.
   b. Add an appropriate volume of the prepared activated sludge to give a concentration of suspended solids of not more than 30 mg/L in the final 3 liters of inoculated mixture. Alternatively, first dilute the prepared sludge to give a suspension of 500-1000 mg/L in the mineral medium before adding an aliquot to the contents of the 5-liter flask to attain a concentration of 30 mg/L.
   c. Aerate these inoculated mixtures with CO_2-free air overnight to purge the system of carbon dioxide.
   d. Add the test material and reference compound, separately, as known volumes of stock solutions, to replicate flasks to yield concentrations, contributed by the added chemicals, of 10 – 20 mg DOC or TOC per liter. Leave some flasks without addition of chemicals as inoculum controls. Add poorly soluble test substances directly to the flasks on a weight or volume basis. Make up the volumes of suspensions in all flasks to 3 liters by the addition of mineral medium previously aerated with CO_2-free air.
   e. If required, use one flask to check the possible inhibitory effect of the test substance by adding both the test and reference substances at the same concentrations as present in the other flasks.
   f. If required, check whether the test substance is degraded abiotically by using a sterilized uninoculated solution of the chemical. Sterilize by the addition of a toxic substance at an appropriate concentration.
   g. If barium hydroxide is used, connect three absorption bottles, each containing 100 mL of 0.0125M barium hydroxide solution, in series to each 5-liter flask. The solution must be free of precipitated sulfate and carbonate and its strength must be determined immediately before use.
   h. If sodium hydroxide is used, connect two traps, the second acting as a control to demonstrate that all the carbon dioxide was absorbed in the first. Absorption bottles fitted with serum bottle closures are suitable. Add 200 mL 0.05M sodium hydroxide to each bottle. This is sufficient to absorb the total quantity of carbon dioxide evolved when the test substance is completely degraded.
   i. In a typical run, the following flasks are used:
      i. Flasks 1 & 2: containing test substance and inoculum (test suspension)
      ii. Flasks 3 & 4: containing only inoculum (inoculum blank)
      iii. Flask 5: containing reference compound and inoculum (procedure control)
      iv. Flask 6: containing test substance and sterilizing agent (abiotic sterile control)
      v. Flask 7: containing test substance, reference compound and inoculum (toxicity control)

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II. Start the test by bubbling CO_2-free air through the suspensions at a rate of 30-100 mL/minute.

III. CO_2 Determination
   a. It is mandatory to follow the CO_2 evolution from the test suspensions and inoculum blanks in parallel and it is advisable to do the same for the other test vessels.
   b. During the first ten days it is recommended that analyses of CO_2 should be made every second or third day and then at least every fifth day until the 28th day so that the 10-day window period can be identified. On the days of CO_2 measurement, disconnect the barium hydroxide absorber closest to the test vessel and titrate the hydroxide solution with 0.05M HCl using phenolphthalein as the indicator. Move the remaining absorbers one place closer to the test vessel and place a new absorber containing 100 mL fresh 0.0125M barium hydroxide at the far end of the series. Make titrations are needed (for example, when substantial precipitation is seen in the first trap and before any is evident in the second, or at least weekly). Alternatively, with NaOH as absorbent, withdraw a sample of the sodium hydroxide solution from the absorber nearest to the test vessel using a syringe. The sample volume needed will depend on the carbon analyzer used, but sampling should not significantly change the absorbent volume over the test period. Inject the sample into the IC part of the carbon analyzer for analysis of evolved carbon dioxide directly. Analyze the contents of the second trap only at the end of the test in order to correct for any carry-over of carbon dioxide.
   c. On the 28th day withdraw samples, optionally, for DOC and/or specific chemical analysis. Add 1 mL of concentrated hydrochloric acid to each test vessel and aerate them overnight to drive off the carbon dioxide present in the test suspensions. On day 29 make the last analysis of evolved carbon dioxide.

Data and Reporting

I. Treatment of Results
   a. Data from the test should be entered onto the attached data sheet.
   b. The amount of CO_2 produced is calculated from the amount of base remaining in the absorption bottle. When 0.0125M $Ba(OH)_2$ is used as the absorbent, the amount remaining is assessed by titrating with 0.05M HCl.
   c. Since 1 mmol of CO_2 is produced for every mol of $Ba(OH)_2$ reacted to $BaCl_2$ and 2 mmol of HCl are needed for the titration of the remaining $Ba(OH)_2$ and given that the molecular weight of CO_2 is 44 g, the weight of CO_2 produced (in mg) is calculated by:

$$\frac{0.05 \times (50 - mL\ HCl\ Titrated) \times 44}{2} = 1.1 \times (50 - mL\ HCl\ Titrated)$$

Therefore, the factor to convert volume of HCl titrated to mg CO_2 produced is 1.1 in this case. Calculate the weights of CO_2 produced from the inoculum alone and from the inoculum plus test substance using the respective titration values. The difference is the weight of CO_2 produced from the test substance alone.

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d. The percentage biodegradation is calculated from:

$$\%\ Degradation = \frac{mg\ CO_2\ Produced}{ThCO_2 \times mg\ Test\ Substance\ Added} \times 100$$

Or

$$\%\ Degradation = \frac{mg\ CO_2\ Produced}{mg\ TOC\ Added\ in\ Test\ \times 3.67} \times 100$$

$$Where\ 3.67\ is\ the\ conversion\ factor\ \left(\frac{44}{12}\right)\ for\ carbon\ to\ carbon\ dioxide$$

e. When NaOH is used as the absorbent, calculate the amount of CO_2 produced after any time interval from the concentration of inorganic carbon and the volume of absorbent used. Calculate the percentage degradation from:

$$\%\ ThCO_2 = \frac{mg\ IC\ from\ Test\ Flask - mg\ IC\ from\ Blank}{mg\ TOC\ Added\ as\ Test\ Substances} \times 100$$

f. Display the course of degradation graphically and indicate the 10-day window. Calculate and report the percentage removal achieved at the plateau, at the end of the test, and/or at the end of the 10-day window, whichever is appropriate.
g. When appropriate, calculate DOC removals using the equation given in 301 A paragraph 27.
h. When an abiotic control is used, calculate the percentage abiotic degradation by:

$$\%\ Abiotic\ Degradation = \frac{CO_2\ Produced\ by\ Sterile\ Flask\ After\ 28\ Days\ (mg)}{ThCO_2\ (mg)} \times 100$$

Validity of Tests

I. The IC content of the test substance suspension in the mineral medium at the beginning of the test must be less than 5% of the TC, and the total CO_2 evolution in the inoculum blank at the end of the test should not normally exceed 40 mg/L medium. If values greater than 70 mg CO_2/L are obtained, the data and experimental technique should be examined critically.

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OECD 301B Ready Biodegradability Assay

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Data Sheet

Laboratory	Active Micro Technologies Tissue Culture Laboratory		
Test Start Date	12/29/2014		
Test Substance	**Name**	Leucidal® Liquid PT	
	Stock Solution Concentration	2 g/L	
	Initial Concentration in Medium	20 mg/L	
Inoculum	**Source**	Activated Sludge	
	Treatment Given	Centrifugation	
	Pre-conditioning	N/A	
	Suspended Solids Concentration in Reaction Mixture	4 mg/L	
Reference Material	Sodium Benzoate	**Concentration**	20 mg/L
CO_2 Production and Degradability	**Method**	**$Ba(OH)_2$**	0.0125M
		NaOH	N/A
		Other	N/A
Total Contact Time	28 Days		
Total CO_2 Evolved Measurements	**Days**	2, 4, 11, 17, 23, 28	
Degradation Over Time	88.3%		
Remarks	Test material was readily biodegradable		
Conclusion	This test met the criteria for a valid assay		

Discussion

Based on the testing conducted in accordance with the specified method, test **Leucidal® Liquid PT** achieved 88.3% biodegradation after 28 days of testing. The product met method requirements for Readily Biodegradability classification.

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OECD TG 442C: *In Chemico* Skin Sensitization

Tradename: Leucidal® Liquid PT

Code: M15021

CAS #: 1686112-36-6 (or) 9015-54-7

Test Request Form #: 1421

Lot #: 40974P

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
OECD TG 442C: *In Chemico* Skin Sensitization
Direct Peptide Reactivity Assay (DPRA)

Introduction

A skin sensitizer is a substance that will lead to an allergic response following skin contact[1]. Haptenation is the covalent binding of a hapten, or low-molecular weight substance or chemical, to proteins in the skin. This is considered the prominent mechanism which defines a chemical as a sensitizer. Haptenation is described as a "molecular initiating event" in the OECD Adverse Outcome Pathway (AOP) for skin sensitization which summarizes the key events known to be involved in chemically-induced allergic contact dermatitis[2]. The direct peptide reactivity assay (DPRA) is designed to mimic the covalent binding of electrophilic chemicals to nucleophilic centers in skin proteins by quantifying the reactivity of chemicals towards the model synthetic peptides containing cysteine and lysine. The DPRA is able to distinguish sensitizers from non-sensitizer with 82% accuracy (sensitivity of 76%; specificity of 92%)[3].

This assay was conducted to determine skin sensitization hazard of **Leucidal® Liquid PT** in accordance with European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) and OECD Test Guideline 442C.

Assay Principle

The DPRA is an *in chemico* method which addresses peptide reactivity by measuring depletion of synthetic heptapeptides containing either cysteine or lysine following 24 hours incubation with the test substance. The peptide is a custom material containing phenylalanine to aid in detection. Depletion of the peptide in the reaction mixture is measured by HPLC with gradient elution and UV detection at 220 nm. Cysteine and lysine peptide percent depletion values are then calculated and used in a prediction model which allows assigning the test chemical to one of four reactivity classes used to support the discrimination between sensitizers and non-sensitizers.

1. United Nations Economic Commission (UNECE) (2013) Global Harmonized System of Classification and Labelling of Chemicals (GHS) 5th Revised Edition
2. OECD (2012). The Adverse Outcome Pathway for Skin Sensitization Initiated by Covalent Binding to Proteins. Part 1: Scientific Evidence. Series on Testing and Assessment No. 168
3. EC EURL ECVAM (2012) Direct peptide reactivity assay (DPRA) validation study report; pp 1 -74.

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Materials

A. Equipment:	HPLC-UV (Waters Breeze - Waters 2998 Photodiode Array Detector); Pipettes; Analytical balance
B. HPLC/Guard Columns:	Agilent Zorbax SB-C18 2.1mm x 100mm x 3.5µm; Phenomenex Security Guard C18 4mm x 2mm
C. Chemicals:	Trifluoroacetic acid; Ammonium acetate; Ammonium hydroxide; Acetonitrile; Cysteine peptide (Ac-RFAA**C**AA-COOH); Lysine peptide (Ac-RFAA**K**AA-COOH); Cinnamic aldehyde
D. Reagents/Buffers:	Sodium phosphate buffer (100mM); Ammonium acetate buffer (100mM)
E. Other:	Sterile disposable pipette tips

Methods

Solution Preparation:

- 0.667mM Cysteine Peptide in 100mM Phosphate Buffer (pH 7.5)
- 0.667mM Lysine Peptide in 100mM Ammonium Acetate Buffer (pH 10.2)
- 100mM Cinnamic Aldehyde in Acetonitrile
- 100mM **Leucidal® Liquid PT** in Acetonitrile

Reference Controls:

- Reference Control A: For calibration curve accuracy
- Reference Control B: For peptide stability over analysis time of experiment
- Reference Control C: For verification that the solvent does not impact percent peptide depletion

Sample, Reference Control, and Co-Elution Control Preparation:

- Once these solutions have been made they should be incubated at room temperature, protected from light, for 24±2 hours before running HPLC analysis.
- Each chemical should be analyzed in triplicate.

1:10 Ratio, Cysteine Peptide 0.5mM Peptide, 5mM Test Chemical	1:50 Ratio, Lysine Peptide 0.5mM Peptide, 25mM Test Chemical
• 750µL Cysteine Peptide Solution (or 100mM Phosphate Buffer, pH 7.5, for Co-Elution Controls) • 200µL Acetonitrile • 50µL Test Chemical Solution (or Acetonitrile for Reference Controls)	• 750µL Lysine Peptide Solution (or 100mM Ammonium Acetate Buffer, pH 10.2, for Co-Elution Controls) • 250µL Test Chemical Solution (or Acetonitrile for Reference Controls)

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Calibration Curve:

- Standards are prepared in a solution of 20% Acetonitrile:Buffer
  - For the Cysteine peptide using the phosphate buffer, pH 7.5
  - For the Lysine peptide using the ammonium acetate buffer, pH 10.2

	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
mM Peptide	0.534	0.267	0.1335	0.0667	0.0334	0.0167	0.000

HPLC Analysis:

- HPLC-UV system should be equilibrated at 30°C with 50% Mobile Phase A (0.1% (v/v) trifluoroacetic acid in water) and 50% Mobile Phase B (0.085% (v/v) trifluoroacetic acid in acetonitrile) for 2 hours
- Absorbance is measured at 220nm
- Flow Conditions:

Time	Flow	%A	%B
0 minutes	0.35 mL/min	90	10
10 minutes	0.35 mL/min	75	25
11 minutes	0.35 mL/min	10	90
13 minutes	0.35 mL/min	10	90
13.5 minutes	0.35 mL/min	90	10
20 minutes	End Run		

Data and Reporting

Acceptance Criteria:

1. The following criteria must be met for a run to be considered valid:
   a. Standard calibration curve should have an $r^2 > 0.99$.
   b. Mean percent peptide depletion values of three replicates for the positive control cinnamic aldehyde should be between 60.8% and 100% for the cysteine peptide and between 40.2% and 69% for the lysine peptide and the maximum standard deviation should be <14.9 for the percent cysteine depletion and <11.6 for the percent lysine depletion.
   c. Mean peptide concentration of reference controls A should be 0.50±0.05mM and the coefficient of variable of the peptide peak areas for reference B and C in acetonitrile should be <15.0%.

2. The following criteria must be met for a test chemical's results to be considered valid:
   a. Maximum standard deviation should be <14.9 for percent cysteine depletion and <11.6 for percent lysine depletion.
   b. Mean peptide concentration of the three reference control C should be 0.50±0.05mM.

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Prediction Model:

Cysteine 1:10/Lysine 1:50 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
0% < Mean % Depletion < 6.38%	Minimal Reactivity	Non-sensitizer
6.38% < Mean % Depletion < 22.62%	Low Reactivity	Sensitizer
22.62% < Mean % Depletion < 42.47%	Moderate Reactivity	Sensitizer
42.47% < Mean % Depletion < 100%	High Reactivity	Sensitizer

If co-elution occurs with the lysine peptide, than the cysteine 1:10 prediction model can be used:

Cysteine 1:10 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
0% < Cys % Depletion < 13.89%	Minimal Reactivity	Non-sensitizer
13.89% < Cys % Depletion < 23.09%	Low Reactivity	Sensitizer
23.09% < Cys % Depletion < 98.24%	Moderate Reactivity	Sensitizer
98.24% < Cys % Depletion < 100%	High Reactivity	Sensitizer

Results and Discussion

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated.

Percent peptide depletion is determined by the following equation:

$$Percent\ Peptide\ Depletion = \left[1 - \left(\frac{Peptide\ Peak\ Area\ in\ Replicate\ Injection}{Mean\ Peptide\ Peak\ Area\ in\ Reference\ Controls\ C}\right)\right] \times 100$$

Based on HPLC-UV analysis of **Leucidal® Liquid PT (code M15021)** we can determine that this product is not a sensitizer and will not cause allergic contact dermatitis. The Mean Percent Depletion of Cysteine and Lysine was 2.43% causing minimal reactivity in the assay giving us the prediction of a non-sensitizer.

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OECD TG 442D: *In Vitro* Skin Sensitization

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Tradename: Leucidal® Liquid PT

Code: M15021

CAS #: 1686112-36-6 (or) 9015-54-7

Test Request Form #: 1422

Lot #: 40974P

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
OECD TG 442D: In Vitro Skin Sensitization
ARE-Nrf2 Luciferase Test Method

Introduction

Skin sensitization refers to an allergic response following skin contact with the tested chemical, as defined by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals[1]. Substances are classified as skin sensitizers if there is evidence in humans that the substance can lead to sensitization by skin contact or positive results from appropriate tests, both *in vivo* and *in vitro*. Utilization of the KeratinoSens™ cell line allows for valid *in vitro* testing for skin sensitization.

This assay was conducted to determine skin sensitization potential of **Leucidal® Liquid PT** in accordance with the UN GHS.

Assay Principle

The ARE-Nrf2 luciferase test method addresses the induction of genes that are regulated by antioxidant response elements (ARE) by skin sensitizers. The Keap1-Nrf2-ARE pathways have been shown to be major regulator of cytoprotective responses to oxidative stress or electrophilic compounds. These pathways are also known to be involved in the cellular processes in skin sensitization. Small electrophilic substances such as skin sensitizers can act on the sensor protein Keap1 (Kelch-like ECH-associated protein 1), by covalent modification of its cysteine residue, resulting in its dissociation from the transcription factor Nrf2 (nuclear factor-erythroid 2-related factor 2). The dissociated Nrf2 can then activate ARE-dependent genes such as those coding for phase II detoxifying enzymes.

The skin sensitization assay utilizes the KeratinoSens™ method which uses an immortalized adherent human keratinocyte cell line (HaCaT cell line) that has been transfected with a selectable plasmid to quantify luciferase gene induction as a measure of activation of Keap1-Nrf2-antioxidant/electrophile response element (ARE). This test method has been validated by independent peer review by the EURL-ECVAM. The addition of a luciferin containing reagent to the cells will react with the luciferase produced in the cell resulting in luminescence which can be quantified with a luminometer.

1. United Nations (UN) (2013). Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Fifth revised edition, UN New York and Geneva, 2013

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Materials

A.	**Incubation Conditions:**	37°C at 5% CO_2 and 95% relative humidity (RH)
B.	**Equipment:**	Humidified incubator; Biosafety laminar flow hood; Microplate Reader; Pipettes
C.	**Cell Line:**	KeratinoSens™ by Givaudan Schweiz AG
D.	**Media/Buffers:**	Dulbecco's Modified Eagle Medium (DMEM); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS); Geneticin
E.	**Culture Plate:**	Flat bottom 96-well tissue culture treated plates
F.	**Reagents:**	Dimethyl Sulfoxide (DMSO); Cinnamic Aldehyde; ONE-Glo Reagent; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); sodium lauryl sulfate (SLS)
G.	**Other:**	Sterile disposable pipette tips; wash bottles

Methods

KeratinoSens™ were into seeded four 96-well tissue culture plates and allowed to grow to 80 – 90% confluency in DMEM containing 10% FBS and 500µg/mL G418 geneticin. Twelve test concentrations of **Leucidal® Liquid PT** were prepared in DMSO with a concentration range from 0.98 – 2000 µM. These 12 concentrations were assayed in triplicate in 2 independently performed experiments. The positive control was cinnamic aldehyde for which a series of 5 concentrations prepared in DMSO had final test concentrations of 4 – 64µM. The negative control was a 1% test concentration of DMSO.

24 hour post KeratinoSens™ seeding, the culture media was removed and replaced with fresh media containing 10% FBS without G418 geneticin. 50 µL of the above described test concentrations was added to the appropriate wells. The treated plates were then incubated for 48 hours at 37°C in the presence of 5% CO_2 and 95% relative humidity. After treatment incubation was complete the media was removed and the wells were washed with PBS 3 times.

One of the four plates was used for a cytotoxicity endpoint, where MTT was added to the wells and incubated for 4 hours at 37°C in the presence of 5% CO_2. SLS was then added to the wells and incubated overnight at room temperature. A spectrometer measured the absorbance at 570 nm. The absorbance values (optical density) were then used to determine the viability of each well by comparing the optical density of each test material treated well to that of the solvent control wells to determine the IC_{50} and IC_{30} values.

The remaining 3 plates were used in the luciferase induction endpoint of the assay. 100 µL of Promega's ONE-Glo Reagent was added to 100 µL of fresh media containing 10% FBS without geneticin. Cells were incubated for 5 minutes to induce cell lysis and release luciferin into the media. Plates were read with a luminometer and $EC_{1.5}$ and maximum response (I_{max}) values were obtained.

Data and Reporting

Acceptance Criteria:

1. Gene induction obtained with the positive control, cinnamic aldehyde, should be statistically significant above the threshold of 1.5 in at least one of the tested concentrations (from 4 to 64 µM).
2. The EC1.5 value should be within two standard deviations of the historical mean and the average induction in the three replicates for cinnamic aldehyde at 64 µM should be between 2 and 8.
3. The average coefficient of variability of the luminescence reading for the negative (solvent) control DMSO should be below 20% in each experiment.

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A KeratinoSens™ prediction is considered positive if the following conditions are met:

1. The Imax is higher than 1.5-fold and statistically significantly higher as compared to the solvent (negative) control
2. The cellular viability is higher than 70% at the lowest concentration with a gene induction above 1.5 fold (i.e., at the EC1.5 determining concentration)
3. The $EC_{1.5}$ value is less than 1000 µM (or < 200 µg/ml for test chemicals with no defined MW)
4. There is an apparent overall dose-response for luciferase induction

Results

Compound	Classification	$EC_{1.5}$ (µM)	IC_{50}	I_{max}
Cinnamic aldehyde	Sensitizer	19	289.19 µM	31.6
DMSO	Non-Sensitizer	No Induction	243.24 µM	1.2
Leucidal® Liquid PT	Non-Sensitizer	No Induction	> 1000 µM	0.6

Table 1: Overview of KeratinoSens™ Assay Results

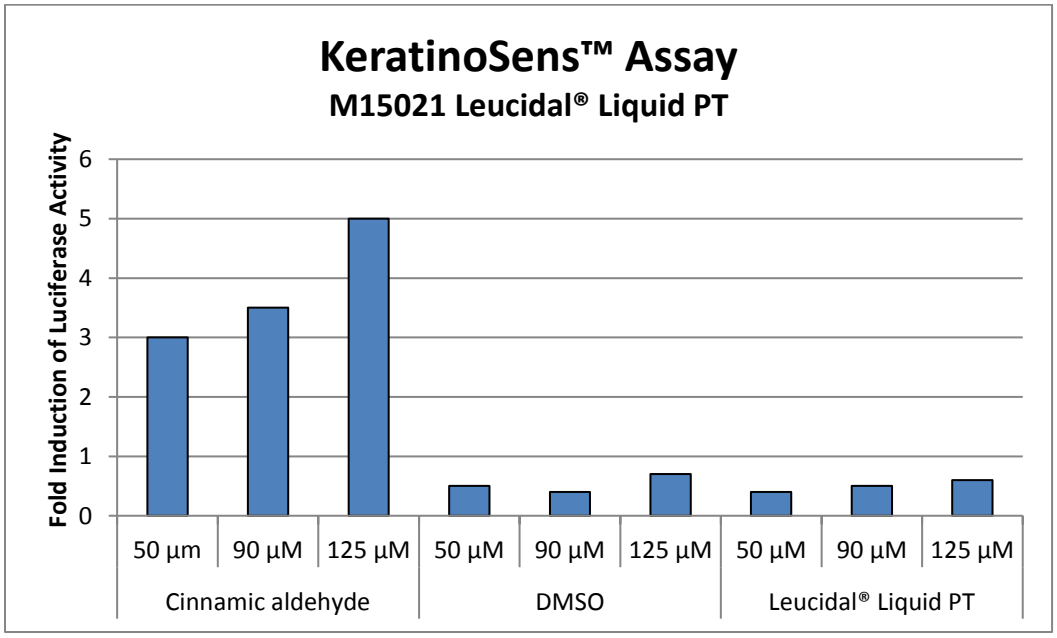

Figure 1: Fold Induction of Luciferase

Discussion

As shown in the results, **Leucidal® Liquid PT (code M15021)** was not predicted to be a skin sensitizer based on the KeratinoSens™ ARE-Nrf2 Luciferase Test Method as there was not a significant increase in luciferase expression. It can be concluded that **Leucidal® Liquid PT** can be safely used in cosmetics and personal care products at typical use levels.

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Date Issued: August 31, 2015

ALLERGEN DECLARATION

RE: *Leucidal® Liquid PT (M15021)*

Please be advised that this form is to certify that the above referenced product, manufactured at Active Micro Technologies, LLC, does not contain any of the allergens listed below:

Eggs – or egg products

Milk – or milk products (includes whey, lactose, casein, milk, cream)

Peanuts – or peanut products

Fish – (includes fish: surimi, cod, pollack, whitefish)

Shellfish – (shrimp, lobster, crab, clams, etc.)

Soybeans – or soybean products (includes soya powder, protein, oil, lecithin, tofu)

Wheat – or wheat products (includes Gluten)

Tree nuts – (almond, brazil nut, cashew, chestnut, hazelnut, filbert, pine nuts (pinyon, pinon), pistachio, pecan, macadamia, walnut).

Palm Oil – or palm kernel oil

Corn – or corn products

If you have any further questions or concerns, please contact us at: 1-704-276-7100

Heavy Metals Statement

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February 28, 2018

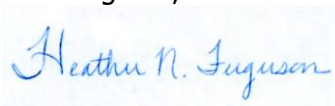
To Whom It May Concern,

This letter is to certify that Leucidal® Liquid PT (M15021) has the following heavy metals profile:

Heavy Metals:	Less than 20 ppm
Lead:	Less than 10 ppm
Antimony:	Less than 5 ppm
Arsenic:	Less than 2 ppm
Mercury:	Less than 1 ppm
Cadmium:	Less than 1 ppm

**Please note: The above levels illustrate the Maximum Limits. Values for Antimony and Mercury do not appear on the Specification for Leucidal® Liquid PT.

Best Regards,



Tomorrow's Vision... *Today!* ®

Heather Ferguson |R&D Coordinator

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Bacterial Reverse Mutation Test

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Test Article: Leucidal® Liquid PT
Code Number: M15021
CAS #: 68333-16-4

Sponsor:
Active Micro Technologies, LLC
107 Technology Drive
Lincolnton, NC 28092

Study Director: *Erica Segura*
Principle Investigator: *Monica Beltran*

Test Performed:
Genotoxicity: Bacterial Reverse Mutation Test

Reference:
OECD471/ISO10993.Part 3

Test Request Number: 1020

SUMMARY

A *Salmonella typhimurium/Escherichia coli* reverse mutation standard plate incorporation study described by Ames *et al.* (1975) was conducted to evaluate whether a test article solution **Leucidal® Liquid PT** would cause mutagenic changes in the average number of reveratants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535 and tryptophan-dependent *Escherichia coli* strain WP2*uvr*A in the presence and absence of Aroclor-induced rat liver S9. This study was conducted to satisfy, in part, the Genotoxicity requirement of the International Organization for Standardization: Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

The stock test article was tested at eight doses levels along with appropriate vehicle control and positive controls with overnight cultures of tester strains. The test article solution was found to be noninhibitory to growth of tester strain TA98, TA100, TA1537, TA1535 and WP2*uvr*A after Sport Inhibition Screen.

Separate tubes containing 2 ml of molten top agar at 45^0C supplemented with histidine-biotin solution for the *Salmonella typhimurium* strains and supplemented with tryptophan for *Escherichia coli* strain were inoculated with 100 µl of tester strains, 100 µl of vehicle or test article dilution were added and 500 µl aliquot of S9 homogenate, simulating metabolic activation, was added when necessary. After vortexing, the mixture was poured across the Minimal Glucose Agar (GMA) plates. Parallel testing was also conducted with positive control correspond to each strain, replacing the test article aliquot with 50µl aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for 48 hours at 37^0C. The mean numbers of revertants of the test plates were compared to the mean number of revertants of the negative control plates for each of the strains tested. The means obtained for the positive controls were used as points of reference.

Under the conditions of this assay, the test article solution was considered to be Non-Mutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* tester strain WP2*uvr*A. The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 100993, Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicology.

All *Salmonella* tester strain cultures demonstrated the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene. Cultures of tester strains TA98 and TA100 demonstrated the presence of the Pkm101 plasmid R-factor. All WP2 *uvr*A cultures demonstrated the deletion in the *uvr*A gene. All cultures demonstrated the characteristic mean number of spontaneous revertants in the vehicle controls as follows: TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21, WP2*uvr*A, 10-60.

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I. Introduction

A. **Purpose**
A *Salmonella typhimurium/Escherichia coli* reverse mutation standard plate incorporation study was conducted to evaluate whether a test article solution would cause mutagenic changes in the average number of revertants for *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2*uvr*A in the presence and absences of the S9 metabolic activation. Bacterial reverse mutation tests have been widely used as rapid screening procedures for the determination of mutagenic and potential carcinogenic hazards.

II. Materials

A. **Storage Conditions:** Room temperature (23-25C).
B. **Vehicle:** Sterile DI Water.
C. **Preparation:** Eight different doses level were prepared immediately before use with sterile DI water.
D. **Solubility/Stability:** 100% Soluble and Stable.
E. **Toxicity:** No significant inhibition was observed.

III. Test System

A. Test System

Each *Salmonella typhimurium* and *Escherichia coli* tester strain contains a specific deep rough mutation (*rfa)*, the deletion of *uvr*B gene and the deletion in the *uvr*A gene that increase their ability to detect mutagens, respectively. These genetically altered *Salmonella typhimurium* strains (TA98, TA100, TA1537 and TA1535) and *Escherichia coli* strain (WP2*uvr*A) cannot grow in the absence of histidine and tryptophan, respectively. When placed in a histidine-tryptophan free medium, only those cells which mutate spontaneously back to their wild type states are able to form colonies. The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant, but if a mutagen is added to the test system, the mutation rate is significantly increased.

Tester strain	Mutations/Genotypic Relevance
TA98	hisD3052, Dgal chlD bio *uvr*B *rfa* pKM101
TA100	hisG46, Dgal chlD BIO *uvr*B *rfa* pKM101
TA1537	hisC3076, *rfa*, Dgal chlD bio *uvrB*
TA 1535	hisG46, Dgal chlD bio *uvr*B *rfa*
WP2*uvr*A	trpE, *uvr*A

rfa	=	causes partial loss of the lip polysaccharide wall which increases permeability of the cell to large molecules.
uvrB	=	deficient DNA excision-repair system (i.e., ultraviolet sensitivity)
pKM101	=	plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens.
*uvr*A	=	All possible transitions and transversions, small deletions.

B. Metabolic Activation

Aroclor induced rat liver (S9) homogenate was used as metabolic activation. The S9 homogenate is prepared from male Sprague Dawley rats. Material is supplied by MOLTOX, Molecular Toxicology, Inc.

C. Preparation of Tester strains

Cultures of *Salmonella typhimurium* TA98, TA100,TA1537, TA1535 and *Escherichia coli* WP2uvrA were inoculated to individual flasks containing Oxoid broth No.2. The inoculated broth cultures were incubated at 37°C in an incubator shaker operating at 140-150 rpm for 12-16 hours.

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D. Negative Control
Sterile DI water (vehicle without test material) was tested with each tester strain to determine the spontaneous reversion rate. Each strain was tested with and without S9 activation. These data represented a base rate to which the number of reveratants colonies that developed in each test plate were compared to determine whether the test material had significant mutagenic properties.

E. Positive Control
A known mutagen for each strain was used as a positive control to demonstrate that tester strains were sensitive to mutation to the wild type state. The positive controls are tested with and without the presence of S9 homogenate.

F. Titer of the Strain Cultures:
Fresh cultures of bacteria were grown up to the late exponential or early stationary phase of growth; to confirm this, serial dilutions from each strain were conducted, indicating that the initial population was in the range of 1 to $2x10^9$/ml.

IV. Method

A. Standard Plate Incorporation Assay:
Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the *Salmonella typhimurium* and tryptophan for *Escherichia coli* were inoculated with 100 µl of culture for each strain and 100 µl of testing solution or vehicle without test material. A 500 µl aliquot of S9 homogenate, simulating metabolic activation, was added when necessary. The mixture was poured across Minimal Glucose Agar plates labeled with strain number and S9 activation (+/-). When plating the positive controls, the test article aliquot was replaced by 50µl aliquot of appropriate positive control. The test was conducted per duplicate. The plates were incubated for 37°C for 2 days. Following the incubation period, the revertant colonies on each plate were recorded. The mean number of reveratnts was determined. The mean numbers of revertants of the test plates were compared to the mean number of reverants of the negative control of each strain used.

V. Criteria for a Valid Test

For the test solution to be evaluated as a test failure or “potential mutagen” there must have been a 2-fold or greater increase in the number of mean revertants over the means obtained from the negative control for any or all strains. Each positive control mean must have exhibited at least a 3-fold increase over the respective negative control mean of the *Salmonella* and *Escherichia coli* tester strains used.

All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvr*A cultures must demonstrate the deletion in the *uvr*A gene. All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows: TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21, WP2*uvr*A, 10-60. To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to $0.3x10^9$ cells/ml. The mean of each positive control must exhibit at least 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control. A minimum of three non-toxicdose levels is required to evaluate assay data. A dose level is considered toxic if one of both of the following criteria are met: (1). A >50% reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count. (2). At least a moderate reduction in the background lawn.

VI. Results and Discussion

A. Solubility:
Water was used as a solvent. Solutions from the test article were made from 0.015 to 50mg/ml.

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B. Dose levels tested:
The maximum dose tested was 5000 µg per plate. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate.

C. Titer (Organisms/ml):
5 x 10^8 UFC/ml plate count indicates that the initial population was in the range of 1 to 2 x 10^9 UFC/ml.

D. Standard Plate Incorporation Assay
In no case was there a 2-fold or greater increase in the mean number of revertant testing strains TA98, TA100, TA1537, TA1535 and WP2*uvr*A in the presence of the test solution compared with the mean of vehicle control value. The positive controls mean exhibited at least a 3-fold increase over the respective mean of the *Salmonella typhimurium* and *Escherichia coli* tester strains used. The results are summarized in Appendix 2.

All *Salmonella* tester strain cultures demonstrated the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene. Cultures of tester strains TA98 and TA100 demonstrated the presence of the Pkm101 plasmid R-factor. All WP2 *uvr*A cultures demonstrated the deletion in the *uvr*A gene. All cultures demonstrated the characteristic mean number of spontaneous revertants in the vehicle controls as follows: TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21, WP2*uvr*A, 10-60.

VII. Conclusion

All criteria for a valid study were met as described in the protocol. The results of the Bacterial Reverse Mutation Assay indicate that under the conditions of this assay, the test article solution was considered to be Non-Mutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2*uvr*A.The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 100993, Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicology.

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Appendix 2:

Bacterial Mutation Assay
Plate Incorporation Assay Results

	Concentration µg per Plate	***TA98***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	26	32	26
	1500	22	25	24
	500	28	36	32
	150	39	31	35
	50	25	27	26
	15	21	20	21
	5.0	32	31	30
	1.5	30	22	26
Test Solution w/o S9	5000	15	16	16
	1500	18	21	20
	500	11	19	16
	150	16	18	17
	50	17	16	17
	15	22	25	24
	5.0	20	21	21
	1.5	16	18	17
DI Water w/S9		34	41	38
DI Water w/o S9		21	27	24
2-aminoanthracen w/ S9		398	376	387
2-nitrofluorene w/o S9		245	211	228
Historical Count Positive w/S9		**43-1893**		
Historical Count Positive w/o S9		**39-1871**		
Historical Count Negative w/S9		**4-69**		
Historical Count Negative w/o S9		**3-59**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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	Concentration µg per Plate	***TA100***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	115	125	120
	1500	106	112	109
	500	121	116	119
	150	145	132	139
	50	152	157	155
	15	132	155	144
	5.0	133	142	138
	1.5	138	136	137
Test Solution w/o S9	5000	133	145	139
	1500	115	125	120
	500	132	126	129
	150	117	105	111
	50	110	98	104
	15	125	125	125
	5.0	140	131	136
	1.5	141	125	133
DI Water w/S9		152	165	159
DI Water w/o S9		132	155	144
2-aminoanthracen w/ S9		402	409	406
Sodium azide w/o S9		378	399	389
Historical Count Positive w/S9		**224-3206**		
Historical Count Positive w/o S9		**226-1837**		
Historical Count Negative w/S9		**55-268**		
Historical Count Negative w/o S9		**47-250**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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	Concentration µg per Plate	***TA1537***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	8	8	8
	1500	12	15	14
	500	13	11	12
	150	19	17	18
	50	18	15	17
	15	12	12	12
	5.0	13	15	14
	1.5	11	5	8
Test Solution w/o S9	5000	21	22	22
	1500	25	16	21
	500	12	15	14
	150	11	12	12
	50	15	22	19
	15	16	17	17
	5.0	14	13	14
	1.5	15	8	12
DI Water w/S9		11	19	15
DI Water w/o S9		18	16	17
2-aminoanthracen w/ S9		378	355	367
2-aminoacridine w/o S9		365	389	377
Historical Count Positive w/S9		**13-1934**		
Historical Count Positive w/o S9		**17-4814**		
Historical Count Negative w/S9		**0-41**		
Historical Count Negative w/o S9		**0-29**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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	Concentration µg per Plate	***TA1535***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	19	18	19
	1500	26	27	27
	500	21	28	25
	150	24	20	22
	50	22	30	26
	15	21	21	21
	5.0	20	21	21
	1.5	18	25	22
Test Solution w/o S9	5000	39	42	41
	1500	38	33	36
	500	45	40	43
	150	40	21	31
	50	23	26	25
	15	25	38	32
	5.0	22	24	23
	1.5	20	26	23
DI Water w/S9		18	21	20
DI Water w/o S9		31	30	31
2-aminoanthracen w/ S9		254	288	271
Sodium azide w/o S9		381	345	363
Historical Count Positive w/S9		**22-1216**		
Historical Count Positive w/o S9		**47-1409**		
Historical Count Negative w/S9		**1-50**		
Historical Count Negative w/o S9		**1-45**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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	Concentration µg per Plate	***WP2uvrA***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	15	16	16
	1500	25	28	27
	500	24	24	24
	150	28	25	27
	50	32	35	34
	15	38	31	35
	5.0	38	39	39
	1.5	41	35	38
Test Solution w/o S9	5000	32	36	34
	1500	30	41	36
	500	35	45	40
	150	18	19	19
	50	21	22	22
	15	23	24	24
	5.0	20	15	18
	1.5	41	45	43
DI Water w/S9		42	43	43
DI Water w/o S9		25	35	30
2-aminoanthracen w/ S9		484	492	488
Methylmethanesulfonate w/o S9		401	395	398
Historical Count Positive w/S9		**44-1118**		
Historical Count Positive w/o S9		**42-1796**		
Historical Count Negative w/S9		**8-80**		
Historical Count Negative w/o S9		**8-84**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

Certificate of Origin

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Leucidal® Liquid PT
Code: M15021

Active Micro Technologies, LLC certifies that the above listed ingredient is manufactured in the United States of America.

Active Micro Technologies, LLC certifies that all raw material(s) used to manufacture the above listed ingredient originate in the United States of America.

Active Micro Technologies, LLC certifies that all raw material(s) used to manufacture the above listed ingredient are prepared from non-GMO organisms and are BSE/TSE Free.

Active Micro Technologies, LLC certifies that the above listed ingredient is derived from fermentation using *Lactobacillus*.

Active Micro Technologies, LLC certifies that the above listed ingredient can be classified as Vegan Compliant.

Active Micro Technologies, LLC certifies that the above listed ingredient has never been tested on animals.

Active Micro Technologies, LLC certifies that the above listed ingredient has the following ISO 16128 value, based on the Compositional Breakdown:

Natural Index (NI)	Natural Origin Index (NOI)
1	1

SECTION 1. IDENTIFICATION

Product Name/Identifier	Leucidal® Liquid PT
Product Code	M15021
Recommended Use	Topical Cosmetic Use; Antimicrobial
Restrictions on Use	None
Supplier/Manufacturing Site	Active Micro Technologies, LLC
Address	107 Technology Drive Lincolnton, NC 28092, USA
Telephone No. (24hrs)	1-704-276-7100
Fax No.	1-704-276-7101
Emergency Telephone #	1-704-276-7100 (Mon-Fri: 8:00AM – 5:00PM EST)

SECTION 2. HAZARD(S) IDENTIFICATION

Classification:

GHS / CLP
Basis for Classification: Based on present data no classification and labeling is required according to GHS, taking into account the national implementation (United Nations version 2011)

USA
OSHA Regulatory Status: This material is non-hazardous as defined by the American OSHA Hazard Communication Standard (29 CFR 1910.1200).

Europe
Basis for Classification:
-According to present data no classification and labeling is required according to Reg. (EC) No 1272/2008.
-This product is not classified as hazardous to health or environment according to the CLP regulation.

Labeling Elements:

Pictograph: No hazard symbol expected

Hazard statements/Signal Word: Not applicable

Precautionary statements:
P233: Keep container tightly closed
P281: Use personal protective equipment as required
P402: Store in a dry place
P404: Store in a closed container
P410: Protect from sunlight
P411: Store at temperatures not exceeding 32°C

Other hazards which do not result in classification:

No particular fire or explosion hazard.
By mechanical effect: No particular hazards.
By hygroscopic effect: No particular hazards.

US NFPA 704 (National Fire Protection Association) Hazard Rating System:

Health hazard: Rating 0; Normal Material
Flammability: Rating 0, Will Not Burn
Reactivity: Rating 0, Stable
Other Hazard Information: None

Results of PBT and vPvB assessment:

-PBT: Not applicable
-vPvB: Not applicable

SECTION 3. COMPOSITION / INFORMATION ON INGREDIENTS

Common Chemical Name: Lactobacillus Ferment

Generic name:

Chemical Family: Ferment

Description: Substance

Substance	CAS Numbers	EC Numbers	Percentage
Lactobacillus Ferment	1686112-36-6 (or) 9015-54-7	N/A (or) 295-635-5	100.00%

Formula: Not applicable

SECTION 4. FIRST-AID MEASURES

General: In all cases of doubt, or when symptoms persist, seek medical attention.

Inhalation: Move to fresh air from exposure area. Get medical attention for any breathing difficulty.

Skin contact: Rinse with soap and water. Get medical advice if irritation develops.

Eye contact: Immediately rinse with water for at least 15 minutes, while keeping the eyes wide open. Consult with a physician.

Ingestion:	Consult with a physician.
Protection of first-aiders:	No special protection required.

SECTION 5. FIRE-FIGHTING MEASURES

Fire and explosion hazards:	Not considered to be a fire and explosion hazard
Extinguishing media:	
Suitable:	Water, dry chemicals, foam & carbon dioxide.
Not suitable:	None known
Fire fighting:	Move container from fire area if it can be done without risk. Avoid inhalation of material or combustion by-products. Stay upwind and keep out of low area
Protection for fire-fighters:	Boots, gloves, goggles.

SECTION 6. ACCIDENTAL RELEASE MEASURES

Personal precautions:	Avoid contact with eyes. Personal Protective Equipment: -Protective goggles
Environmental precautions:	Prevent entry into sewers and waterways. Do not allow material to contaminate ground water system
Methods for cleaning up:	
Recovery:	Pick up free liquid for recycling or disposal. Residual liquid can be absorbed on an inert material. Sweep or vacuum up any powder and place in a clearly labeled waste container, avoiding dust formation.
Cleaning/Decontamination:	Wash non-recoverable remainder with water.
Disposal:	For disposal of residues refer to sections 8 & 13.

SECTION 7. HANDLING AND STORAGE

Handling

Technical measures:	Labeling: Keep out of the reach of children.
Measures:	For industrial use, only as directed.
Safe handling advice:	Wash hands after use. Avoid storage near feed or food stuff.

Storage

Technical measures:	Keep container closed.
Recommended Storage Conditions:	Store in a dry place at temperatures not exceeding 32°C. Based on stability studies, the optimum storage temperature for maximization of shelf life is 23 - 25°C. However, it may be stored at temperatures between 16 and 32°C if such specific temperature control is not available. Do not freeze. Please refer to stability data for effects heat or cold may have on the specifications of the product.
Incompatible products:	Avoid contact with strong oxidizers. Refer to the detailed list of incompatible materials (Section 10 Stability/Reactivity)
Packaging:	Product may be packaged in normal commercial packaging.
Packaging materials:	Recommended - Polypropylene & High Density Polyethylene

SECTION 8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Precautionary statements: Ensure adequate ventilation

Control parameters

Occupational exposure Limits:

France:	Not Determined
ACGIH:	Not Determined
Korea:	Not Determined
UK:	Not Determined
Surveillance procedures:	Not Determined
Engineering measures:	Not Determined

Personal Protective Equipment:

Respiratory protection:	Local exhaust
Hand protection:	Protective gloves made of rubber or neoprene.
Eye protection:	Safety glasses.
Collective emergency equipment:	Eye fountain.
Skin and Body Protection:	Suitable protective clothing
Hygiene measures:	Handle in accordance with good industrial hygiene and safety practice.
Measures related to the Environment:	No particular measures.

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance:	Clear to slightly hazy liquid
Color:	Pale yellow to yellow
Odor:	Characteristic

pH (Direct):	7.0 – 8.5
Heavy Metals:	< 20 ppm
Lead:	< 10 ppm
Arsenic:	< 2 ppm
Cadmium:	< 1 ppm
Specific Gravity (25°C):	Not determined
Vapor density:	Not applicable
Boiling Point:	100°C
Freezing Point:	0°C
Melting point:	Not applicable
Flash point:	> 200°F
Oxidizing properties:	Non oxidizing material according to EC criteria.
Solubility:	
In water:	Soluble
In organic solvents:	Not determined
Log P:	Not determined

SECTION 10. STABILITY AND REACTIVITY

Stability: Stable under ordinary conditions of use and storage up to one year then re-test to full product specifications to extend shelf life

Hazardous reactions: None known

Conditions to avoid: No dangerous reactions known under use of normal conditions. Avoid extreme heat.

Materials to avoid: No dangerous reaction known with common products.

Hazardous decomposition products: None known

SECTION 11. TOXICOLOGICAL INFORMATION

Ingestion:	Not Determined
Dermal:	Non-Irritant (Dermal Irritection Model)
Ocular:	Non-Irritant (Ocular Irritection Model)
Inhalation:	Not Determined
Acute toxicity data:	Non-Irritant, Non-Primary Sensitizer & Non-Photo Irritant
Sensitization:	Non-Primary Irritant & Non-Primary Sensitizers; Will not cause allergic contact dermatitis (In Chemico Skin Sensitization Direct Peptide Reactivity Assay & In Vitro Skin Sensitization ARE-Nrf2 Luciferase Test Method)

Repeated dose toxicity: No known effects
Subacute to chronic toxicity: Not Determined

Mutagenicity: Non-Mutagenic (OECD471/ISO10993.Part 3 – Genotoxicity: Bacterial Reverse Mutation Test)

Additional Toxicological Information: This product is not subject to classification according to the calculation method of the General EU Classification Guidelines for Preparations as issued in the latest version.

Specific effects:

Carcinogenicity: No known effects
Mutagenicity: No known effects
Reproductive toxicity: No known effects
Neuro-toxicity: No known effects

For more information: Does not present any particular risk on handling under normal conditions of good occupational hygiene practice.

This product has not been tested for the following:
-Primary cutaneous and corrosive irritation
-Acute oral toxicity

SECTION 12. ECOLOGICAL INFORMATION

Ecotoxicity
Effects on the aquatic environment: EC_{10} (Freshwater Alga): 138.12 mg/L - Not harmful to aquatic organisms
EC_{20} (Freshwater Alga): 240.99 mg/L - Not harmful to aquatic organisms

Biodegradability:
Persistence: Readily Biodegradable (88.3% biodegradation after 28 days of testing)

Bioaccumulation:
Octanol / water partition coefficient: Not Determined

Mobility:
Precipitation:
Expected behavior of the product: Ultimate destination of the product: Soil & sediment.

Other Adverse Effects: None known

SECTION 13. DISPOSAL CONSIDERATIONS

Residues from product

Prohibition: Do not allow the product to be released into the Environment.
Destruction/Disposal: Dispose of in accordance with relevant local regulations

Contaminated packaging

Decontamination/cleaning: Cleaning is not required prior to disposal.
Destruction/Disposal:

Note: Take all necessary precautions when disposing of this product according to local regulations.

SECTION 14. TRANSPORT INFORMATION

UN Number: None
UN Shipping Name: None

Transport Hazard Class: Not classified as dangerous for transport

Land (rail/road): Material is not restrictive for land transport and is not regulated by ADR/RID
Sea: Material is not restrictive for sea transport and is not regulated by IMO/IMDG
Air: Material is not restrictive for air transport and is not regulated by ICAO/IATA

Marine Pollutant: No

Transport/Additional Information: Not regulated for US DOT Transport in non-bulk containers
This material is not dangerous or hazardous

Special Precautions for User: None known

The above regulatory prescriptions are those valid on the date of publication of this sheet. However, given the possible evolution of transport regulations for hazardous materials and in the event of the MSDS in your possession dating back more than 12 months, it is advisable to check their validity with your sales office.

SECTION 15. REGULATORY INFORMATION

Labeling:
EC regulations: This product does not need to be labeled in accordance with EC Directives or respective national laws

Further regulations

United Kingdom: Handle in accordance with relevant British regulation: control of substance Hazardous to Health Regulations Environmental Hygiene Guidance: EH40
Workplace Exposure Limits (revised annually)

Korea regulations:
Industrial safety and hygiene regulation: No
Hazardous material control regulation: No
Fire prevention regulation: No

Other regulations:

EINECS inventory status:	Lactobacillus Ferment	N/A (or) 295-635-5
TSCA inventory status:	Exempt	
AICS inventory status:	Not Listed: 1686112-36-6 Listed: 9015-54-7	
Canadian (CEPA DSL) inventory status:	Not Listed: Lactobacillus Ferment (1686112-36-6) Listed: Protein Hydrolyzates (DSL)	
Japan (MITI list):	Lactobacillus Ferment	
Korea:	Lactobacillus Ferment	
China inventory status:	Lactobacillus Ferment	
Philippines inventory status:	Not Listed: Lactobacillus Ferment (1686112-36-6) Listed: Protein Hydrolyzates	

*Listed on 2010 INCI Standard Chinese Name Directory

Note: The regulatory information given above only indicates the principal regulations specifically applicable to the products described in this sheet. The user's attention is drawn to the possible existence of additional provision which complete these regulations. Please refer to all applicable international, national and local regulations and provisions

SECTION 16. OTHER INFORMATION

Prohibited uses:	For specific uses, food industry, ask the manufacturer for more information.
Last Revision Date:	02/22/2022
Preparation Date:	02/22/2022
MSDS summary of changes	- New Logo - Added Precautionary Statements - Section 2 (Hazards Identification) - Added Heavy Metals & Arsenic – Section 9 (Physical & Chemical Properties) & Updated Transport Information – Section 14 (Transport Information) - Added Acute Toxicity & Mutagenicity Data – Section 11 (Toxicological Information) & Added Biodegradability Data – Section 12 (Ecological Information) - Updated CAS#'s – Section 3 (Composition / Information on Ingredients) & Section 15 (Regulatory Information) - Added Sensitization Data – Section 11 (Toxicological Information) - Updated CAS/EINEC#'s – Section 3 (Composition / Information on Ingredients) & Section 15 (Regulatory Information) & Added Lead & Cadmium – Section 9 (Physical & Chemical Properties), Updated Sensitization & Mutagenicity Data – Section 11 (Toxicological Information) & Updated Biodegradability Data – Section 12 (Ecological Information) - Updated Europe Basis for Classification & Precautionary Statement – Section 2 (Hazards Information), Updated Recovery Methods for Cleaning up – Section 6 (Accidental Release Measures), Updated Recommend Storage Conditions – Section 7 (Handling & Storage)

MSDS summary of changes (Cont.) - Updated Acute Toxicity Data – Section 11 (Toxicological Information) & Added Ecotoxicity Data – Section 12 (Ecological Information)

The information given is based on our knowledge of this product, at the time of publication in good faith. The attention of the user is drawn to the possible risks incurred by using the product for any other purpose other than which it was intended. This is not in any way excuse the user from knowing and applying all the regulations governing their activity. It is sole responsibility of the user to take all precautions required in handling the product. The purpose of mandatory regulation mentioned is to help the user to fulfill his obligations regarding the use of products. This information is not exhaustive, this is not exonerate the user from ensuring that legal obligations other than those mentioned, relating to the use and storage.

Leucidal® Liquid PT Manufacturing Flow Chart

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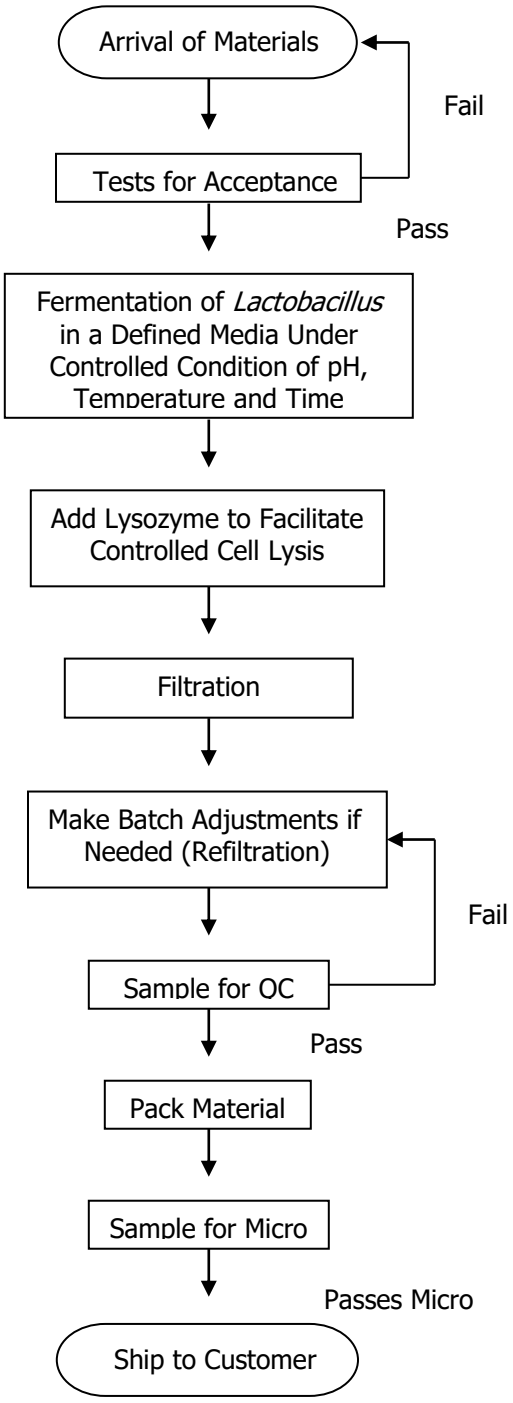

Version#1/01-22-14

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Leucidal® Liquid PT
Certificate of Compliance

Code: M15021
INCI Name: Lactobacillus Ferment
INCI Status: Approved
CAS #: 1686112-36-6 (or) 9015-54-7
EINECS #: N/A (or) 295-635-5

The following information on regulatory clearances is believed to be accurate and is given in good faith as a guide to a global use of our ingredients in cosmetic applications. No representation or warranty as to its competences or accuracy is made. Information is offered for use in general cosmetic applications and may vary in particular applications. Users are responsible for determining the suitability of these products for their own particular use. All regulatory decisions should be made on the advice of your regulatory group or legal counsel.

Country / Regulatory Body	Status of Product
EU (CosIng)	Compliant at Suggested Use Levels
USA (TSCA)	Compliant
Australia (AICS)	Compliant
Japan (METI)	Compliant
Canada (DSL)	Compliant
China (IECIC)	Compliant at Suggested Use Levels
Brazil (ANVISA)	Compliant at Suggested Use Levels
Korea (KECI)	Compliant at Suggested Use Levels
Philippines (PICCS)	Compliant
Mexico (COFEPRIS)	Compliant at Suggested Use Levels

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Leucidal® Liquid PT
Code: M15021

Attention must be paid to the use of this Leucidal® Liquid PT in the equivalent of OTC formulations (eg. quasi-drugs in Japan, or therapeutic goods in Australia). Some countries maintain restricted inventories of raw materials that can be used in those applications so more detailed guidance may be required.

Leucidal® Liquid PT and any components or impurities are in compliance with the rules governing cosmetic products in the European Union (Directive 76/768/ECC & Regulation No. 1223/2009). However, Leucidal® Liquid PT may contain up to 10% water soluble undecylenates. (see also Specification and Compositional Breakdown). This should be borne in mind when formulating products containing Leucidal® Liquid PT as some markets restrict undecylenic acid and it salts at 0.20%. It is therefore recommended that customers consult with their regulatory groups before using Leucidal® Liquid PT at greater than the recommended 2.00% dosage.

Leucidal® Liquid PT is in compliance with the standardized set of rules developed and approved by the NPA (Natural Products Association).

The Nagoya Protocol provides a scheme for the fair and equitable sharing of benefits derived from Genetic Resources. Information regarding the Nagoya Protocol and Access and Benefit Sharing (ABS) is available at https://www.cbd.int/abs/. The agreement focusses on wild taxa and excludes most commercially cultivated crops. For the signatories to the agreement, responsibility for Benefit Sharing falls on the entity exporting or extracting the resource from the signatory country. Active Micro Technologies, LLC audits its suppliers to conform compliance with the Nagoya Protocol where applicable.

Leucidal® Liquid PT is considered a non-hazardous material. All significant toxicological routes of absorption have been considered as well as the systemic effects and margin of safety (MoS) based on a no observed adverse effects level (NOAEL). Due to the restriction placed on animal testing of cosmetic raw materials, and Active Micro Technologies, LLC's internal non-animal testing policy, this product was not tested for NOAEL.

Leucidal® Liquid PT was tested using *in vitro* dermal and ocular irritation models. This product was found to be non-irritating in both models.

To our knowledge the above material is free of CMR (*) substances, as defined according to Regulation (EC) No 1272/2008 and Cosmetic Regulation (EC) No 1223/2009 as amended. Products supported for Personal Care applications will not be classified as CMR (*), as defined by (EC) 1272/2008 on the Classification, Labelling and Packaging of Substances and Mixtures, unless supported by a positive SCCS opinion.

(*) Carcinogenic, Mutagenic, toxic for Reproduction

Active Micro Technologies, LLC certifies that to the best of our knowledge our product does not contain any material listed on California Proposition 65.

Active Micro Technologies, LLC certifies that Leucidal® Liquid PT does not contain any materials prohibited by Halal laws.

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Leucidal® Liquid PT
Code: M15021

Leucidal® Liquid PT is REACH Compliant and free of the following:

- Alcohol
- Butylphenyl methylpropional (Lilial)
- Ethylene Oxide
- Formaldehyde or formaldehyde donors
- Glycol ethers
- Gluten
- Irradiation
- Lactose
- Nanoparticles
- Nitrosamines
- Palm oil/palm kernel oil (or derivatives)
- Parabens
- Paraffin/petroleum products
- Phthalates
- Polyethylene glycol (PEG)
- Residual solvents
- Sulfates
- Sulfites
- Volatile organic compounds

ACTIVE MICRO TECHNOLOGIES

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Raw Component Regulations

Please note that the below are global regulations for the raw materials used to manufacture Leucidal® Liquid PT and are not for the product itself.

Leucidal® Liquid PT may contain up to 10.00% water soluble undecylenates, see below for a list of regulations for Undecylenic Acid and Salts:

Undecylenic Acid and Salts:

- **Europe: Maximum Authorized Concentration of 0.20% (Undecylenic acid)**
- **China: Maximum authorized concentration is 0.20% (Undecylenic acid)**
- **Brazil: Maximum Authorized Concentration of 0.20% (Undecylenic acid)**
- **Mexico: Maximum Authorized Concentration of 0.20% (Undecylenic acid)**
- **Korea: Maximum Authorized Concentration of 0.20% (Undecylenic acid)**
- **ASEAN: Maximum Authorized Concentration of 0.20% (Undecylenic acid)**
- **Mercosur: Maximum Authorized Concentration of 0.20% (Undecylenic acid)**

ATTESTATION OF CONFORMITY
- RAW MATERIALS -
ECOCERT COSMETICS

This attestation has been granted by ECOCERT Greenlife to the company:

ACTIVE MICRO TECHNOLOGIES LLC

107 Technology Drive
LINCOLNTON, NC 28092
UNITED STATES OF AMERICA

whose non-organic raw materials (listed hereafter) have been assessed as compliant to the current version of the ECOCERT standard:

NATURAL AND ORGANIC COSMETICS

This attestation of conformity has been issued on the basis of the terms and conditions for the verification of raw materials according to the ECOCERT standard defining Natural and Organic Cosmetics available on the ECOCERT website: http://www.ecocert.com and the conformity has been established according to the requirements related to the raw materials contained in this standard.

Issued in: L'Isle Jourdain,
the: 16/12/2022,

Emilie CHERHAL
ECOCERT Greenlife General Manager

Valid until: 31/12/2023

ECOCERT Greenlife S.A.S. - Capital 50 000 € - BP 47 - 32600 L'ISLE JOURDAIN - FRANCE
Intra-community VAT n° FR 55 509 534 095
Phone: +33 5 62 07 50 16 - Fax: +33 5 62 07 74 96 - eMail: mpgreenlife@ecocert.com

ATTESTATION OF CONFORMITY - ECOCERT COSMETICS

List of the approved raw materials of: **ACTIVE MICRO TECHNOLOGIES LLC**

Nat: Natural or from natural origin
Veg: Physically processed vegetal ingredients
Synth: Synthetic (petrochemical)

Unless an exception, the following references are published on the ECOCERT raw materials online database for approved raw materials available at the following link: http://ap.ecocert.com/ecoproduits

Commercial name / INCI / Function	%Nat	%Veg	%Synth	Restriction	Approved since
AMTicide Coconut *Lactobacillus (and) Cocos Nucifera (Coconut) Fruit Extract* Skin conditioning, Hair conditioning	100	0	0		01/01/2023
Arborcide OC *Leuconostoc Ferment Filtrate* Skin conditioning, Antimicrobial	100	0	0		01/01/2023
Leucidal Advanced - Aloe *Water (and) Leuconostoc/Aloe Barbadensis Leaf/Sorbus Aucuparia Fruit Ferment Filtrate* Moisturizing, Skin conditioning, Antimicrobial	100	0	0		01/01/2023
Leucidal Advanced - Rowan *Water (and) Leuconostoc/Sorbus Aucuparia Fruit Ferment Filtrate* Emollient, Skin conditioning, Antimicrobial	100	0	0		01/01/2023
Leucidal Liquid *Leuconostoc/Radish Root Ferment Filtrate* Moisturizing, Skin conditioning, Antimicrobial	100	0	0		01/01/2023
Leucidal Liquid AE LFHC *Lactobacillus/Radish Root Ferment Filtrate* Skin conditioning, Antimicrobial	100	0	0		01/01/2023
Leucidal Liquid Complete *Leuconostoc/Radish Root Ferment Filtrate (and) Lactobacillus (and) Cocos Nucifera (Coconut) Fruit Extract* Moisturizing, Skin conditioning, Antimicrobial	100	0	0		01/01/2023

Valid until: 31/12/2023

WARNING: The sole purpose of the present attestation is to allow the raw material(s) to be used in finished products to be certified as compliant to the standard specified in the first page. In no event this attestation should constitute proof of the actual certification of the conformity of the raw material(s) to this standard. In that context, the raw material(s) listed in this attestation must not be qualified and / or marketed as «organic» raw material(s) certified in accordance with the abovementioned standard.
The approval of the raw material (s) listed in the present attestation is personally addressed to the above-mentioned beneficiary. It is the beneficiary's liability to ensure that its own customers are aware of the requirements and prohibitions defined in the terms and conditions and governing any reference to and use of the approval of the raw material(s) and that they abide by it.

ECOCERT Greenlife S.A.S. - Capital 50 000 € - BP 47 - 32600 L'ISLE JOURDAIN - FRANCE
Intra-community VAT n° FR 55 509 534 095
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ATTESTATION OF CONFORMITY - ECOCERT COSMETICS

List of the approved raw materials of: **ACTIVE MICRO TECHNOLOGIES LLC**

Commercial name / INCI / Function	%Nat	%Veg	%Synth	Restriction	Approved since
Leucidal Liquid PT *Lactobacillus Ferment* Skin conditioning, Antimicrobial	100	0	0		01/01/2023
Leucidal Liquid SF *Lactobacillus Ferment* Moisturizing, Skin conditioning, Antimicrobial	100	0	0		01/01/2023
Leucidal Liquid SF (M15019RTZJV) *Leuconostoc/Radish Root Ferment Filtrate* Skin conditioning, Antimicrobial	100	0	0		01/01/2023
Leucidal SF Complete *Lactobacillus Ferment (and) Lactobacillus (and) Cocos Nucifera (Coconut) Fruit Extract* Moisturizing, Skin conditioning, Antimicrobial	100	0	0		01/01/2023
PhytoCide Aspen Bark Extract Powder *Populus Tremuloides Bark Extract* Skin conditioning, Antimicrobial	100	100	0		01/01/2023
PhytoCide Black Currant Powder *Ribes Nigrum (Black Currant) Fruit Extract* Soothing, Skin conditioning, Antimicrobial	100	100	0		01/01/2023
PhytoCide Elderberry OS *Sambucus Nigra Fruit Extract* Skin conditioning, Antimicrobial	100	100	0		01/01/2023

Valid until: 31/12/2023

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Attestation n°: 1550062

ATTESTATION OF CONFORMITY
- RAW MATERIALS -
COSMOS

This attestation has been granted by ECOCERT Greenlife to the company:

ACTIVE MICRO TECHNOLOGIES LLC

107 Technology Drive
LINCOLNTON, NC 28092
UNITED STATES OF AMERICA

whose non-organic raw materials (listed hereafter) have been assessed as compliant to the standard:

COSMOS Version 3 (including all sub-versions)

This attestation of conformity has been issued on the basis of the terms and conditions for the verification of raw materials according to the COSMOS standard available on the COSMOS association website: https://cosmos-standard.org/ and the conformity has been established according to the requirements related to the raw materials contained in this standard.

Issued in: L'Isle Jourdain,
the: 26/04/2023,

Emilie CHERHAL
ECOCERT Greenlife General Manager

Valid until: 31/12/2023

ECOCERT Greenlife S.A.S. - Capital 50 000 € - BP 47 - 32600 L'ISLE JOURDAIN - FRANCE - Intra-community VAT n° FR 55 509 534 095
Phone: +33 5 62 07 50 16 - Fax: +33 5 62 07 74 96 - eMail : mpgreenlife@ecocert.com
Accreditation by COFRAC # 5-0520 for Certification of Products and Services, scope available on www.cofrac.fr.

ATTESTATION OF CONFORMITY - COSMOS

List of the approved raw materials of: **ACTIVE MICRO TECHNOLOGIES LLC**

PPAI: Physically Processed Agro-Ingredients
CPAI: Chemically Processed Agro-Ingredients
NNI: Non Natural Ingredients (Petrochemical origin)
PeMo: Petrochemical Moiety
CSPO: Raw material proceeding from certified sustainable palm/palm kernel oil
Without animal origin: Raw material compliant to the complementary assessment « without animal origin » in force
The asterisk * is used to identify the commercial name of the raw materials concerned by the appendices II and/or V of the Cosmos-standard.
Unless an exception, the following references are published on the ECOCERT raw materials online database for approved raw materials available at the following link: http://ap.ecocert.com/ecoproduits.

Commercial name / INCI / Function	%PPAI	%CPAI	%NNI	%PeMo	Restriction	Approved since
AMTicide Coconut *Lactobacillus (and) Cocos Nucifera (Coconut) Fruit Extract* Skin conditioning, Hair conditioning	0	100	0	0		01/01/2023
AMTicide® VAF *Bacillus Ferment (and) Saccharomyces Ferment Filtrate* Skin Conditioning, Antifungal	0	100	0	0		01/01/2023
Arborcide OC *Leuconostoc Ferment Filtrate* Skin conditioning, Antimicrobial	0	50	0	0		01/01/2023
Leucidal Advanced - Aloe *Water (and) Leuconostoc/Aloe Barbadensis Leaf/Sorbus Aucuparia Fruit Ferment Filtrate* Moisturizing, Skin conditioning, Antimicrobial	0	18	0	0		01/01/2023
Leucidal Advanced - Rowan *Water (and) Leuconostoc/Sorbus Aucuparia Fruit Ferment Filtrate* Emollient, Skin conditioning, Antimicrobial	0	50	0	0		01/01/2023

Valid until: 31/12/2023

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ATTESTATION OF CONFORMITY - COSMOS

List of the approved raw materials of: **ACTIVE MICRO TECHNOLOGIES LLC**

Commercial name / INCI / Function	%PPAI	%CPAI	%NNI	%PeMo	Restriction	Approved since
Leucidal Liquid AE LFHC *Lactobacillus/Radish Root Ferment Filtrate* Skin conditioning, Antimicrobial	0	100	0	0		01/01/2023
Leucidal Liquid Complete *Leuconostoc/Radish Root Ferment Filtrate (and) Lactobacillus (and) Cocos Nucifera (Coconut) Fruit Extract* Moisturizing, Skin conditioning, Antimicrobial	0	64	0	0		01/01/2023
Leucidal Liquid PT *Lactobacillus Ferment* Skin conditioning, Antimicrobial	0	18	0	0		01/01/2023
Leucidal Liquid SF (M15019RTZJV) *Leuconostoc/Radish Root Ferment Filtrate* Skin conditioning, Antimicrobial	0	10	0	0		01/01/2023
Leucidal Liquid SF *Lactobacillus Ferment* Moisturizing, Skin conditioning, Antimicrobial	0	10	0	0		01/01/2023
Leucidal Liquid *Leuconostoc/Radish Root Ferment Filtrate* Moisturizing, Skin conditioning, Antimicrobial	0	50	0	0		01/01/2023
Leucidal SF Complete *Lactobacillus Ferment (and) Lactobacillus (and) Cocos Nucifera (Coconut) Fruit Extract* Moisturizing, Skin conditioning, Antimicrobial	0	32,5	0	0		01/01/2023
Leucidal® Liquid J Max *Leuconostoc/Radish Root Ferment Filtrate (and) Salix Alba (Willow) Bark Extract* Moisturization, Skin/Scalp Conditioning, Antimicrobial	20	30	0	0		01/01/2023

Valid until: 31/12/2023

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ATTESTATION OF CONFORMITY - COSMOS

List of the approved raw materials of: **ACTIVE MICRO TECHNOLOGIES LLC**

Commercial name / INCI / Function	%PPAI	%CPAI	%NNI	%PeMo	Restriction	Approved since
Leucidal® SF Max *Lactobacillus Ferment* Ferment / Skin Conditioning, Antimicrobial	0	25	0	0		01/01/2023
PhytoCide Aspen Bark Extract Powder *Populus Tremuloides Bark Extract* Skin conditioning, Antimicrobial	100	0	0	0		01/01/2023
PhytoCide Black Currant Powder *Ribes Nigrum (Black Currant) Fruit Extract* Soothing, Skin conditioning, Antimicrobial	100	0	0	0		01/01/2023
PhytoCide Elderberry OS *Sambucus Nigra Fruit Extract* Skin conditioning, Antimicrobial	100	0	0	0		01/01/2023
PhytoCide Lichen *2,3-Butanediol (and) Cladonia Rangiferina Extract* Antioxidant, Antimicrobial	0	100	0	0		26/04/2023
ProBiocin V™ *Lactobacillus Ferment Lysate* Antimicrobial, Redness Reduction, Scalp Care	0	100	0	0		01/01/2023

Valid until: 31/12/2023

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Accreditation by COFRAC # 5-0520 for Certification of Products and Services, scope available on www.cofrac.fr.

REACH Compliance Statement

107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

Trade Name: Leucidal® Liquid PT (M15021)

INCI Name: Lactobacillus Ferment

This is to certify that Leucidal® Liquid PT is REACH compliant. Lactobacillus Ferment falls under the polymer exemption.

If you have further questions, please feel free to contact Heather Ferguson at hferguson@activeconceptsllc.com.