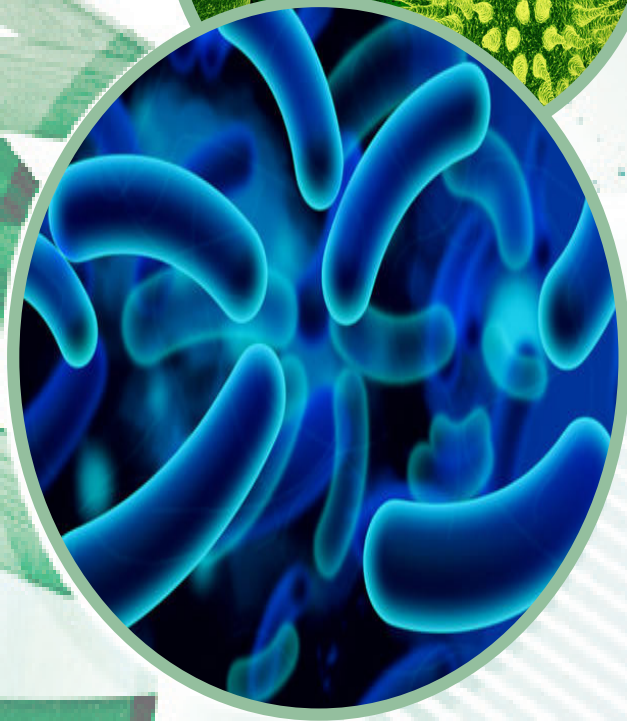


Technical Dossier



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moisture Cosmos condition peptide
Improving solar choice antimicrobial

Leucidal[®] SF Max Powder

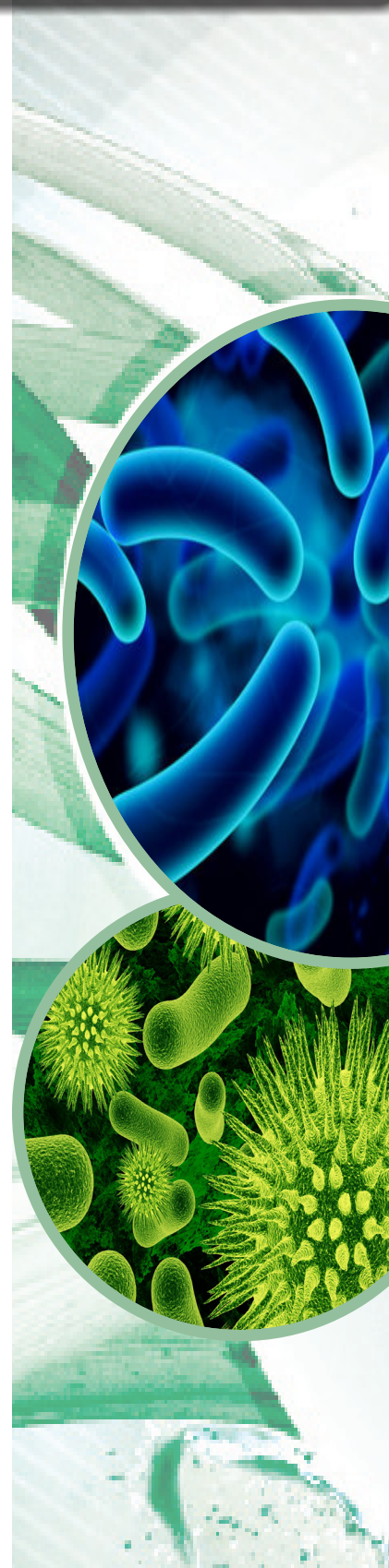
Code Number: M15031

INCI Name: Lactobacillus Ferment

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Leucidal® SF Max Powder

US Patent Number 10,232,197

Technical Data Sheet

BACKGROUND

Consumer preference has continued to drive the personal care industry away from using synthetic preservatives and to focus on using more natural products in their formulations. For these reasons, formulators have been actively searching for alternatives to synthetic materials that can provide broad-spectrum antimicrobial activity and can be added into a wide range of cosmetic applications.

Active Micro Technologies (AMT) has developed a full line of products derived from naturally occurring compounds that provide broad-spectrum antimicrobial protection, in addition to its primary skin and hair care benefits. These novel, natural antimicrobials are considered self-preserving cosmetic actives that are friendly alternatives to synthetic preservatives.

Leucidal® SF Max Powder has proven less is more. This powdered version of the **Leucidal® SF Max** is a more effective, more concentrated version. With five times higher and stronger potency than the original, it allows formulators to save time and money, while simultaneously delivering efficacy with an increased sense of confidence.

SCIENCE

Leucidal® SF Max Powder is a probiotic-based ingredient created by the fermentation of *Lactobacillus* in a defined growth medium. *Lactobacillus* is one of the species of microorganisms used to produce fermented products, such as kimchi, a Korean dietary



staple, and sauerkraut, from cabbage. Like many members of the lactic acid bacteria family, *Lactobacillus* is capable of restricting the growth of other microorganisms by acidifying its environment. However, *Lactobacillus* also produces novel antimicrobial peptides, known as bacteriocins, that are capable of providing broad spectrum antimicrobial protection. During the manufacturing process, lysozyme is added to the ferment filtrate to facilitate a controlled cell lysis to ensure the release of the antimicrobial peptides for maximized activity.

Code Number: M15031

INCI Nomenclature:

Lactobacillus Ferment

INCI Status: Conforms

REACH Status: Fully Compliant

CAS Number: 1686112-36-6 (or)
68333-16-4

EINECS Number: N/A (or) 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: None

Appearance: Free Flowing Powder

Soluble/Miscible: Water Soluble

Suggested Use Levels: 0.50 - 1.00%

Suggested Applications:

Skin Conditioning, Antimicrobial

Leucidal® SF Max Powder

US Patent Number 10,232,197

BENEFITS

A 24-hour skin moisturization study was performed involving 20 M/F participants between the ages of 23-45. Using an untreated control, generic cream base, and an experimental with the same cream base containing 0.5% and 1.0% **Leucidal® SF Max Powder**, moisturization readings were taken and compared. Results from this study are shown below in Figure 1. A Dermalab Corneometer was used to measure moisture levels, in μ Siemens (μ S), as the presence of moisture in the skin improves conductance. Therefore, the higher the conductance measurements, the higher the moisture levels.

As demonstrated by the results of this study, **Leucidal® SF Max Powder** showed high moisturizing capabilities at both concentrations of 0.5% and 1.0%. Please note, each value is an average of three consecutive readings per test site. Based on these results, adding this innovative product provides the formulator the opportunity to capitalize on both the natural antimicrobial properties of **Leucidal® SF Max Powder**, as well as its ability to provide potent moisturizing benefits to the cosmetic formulation.

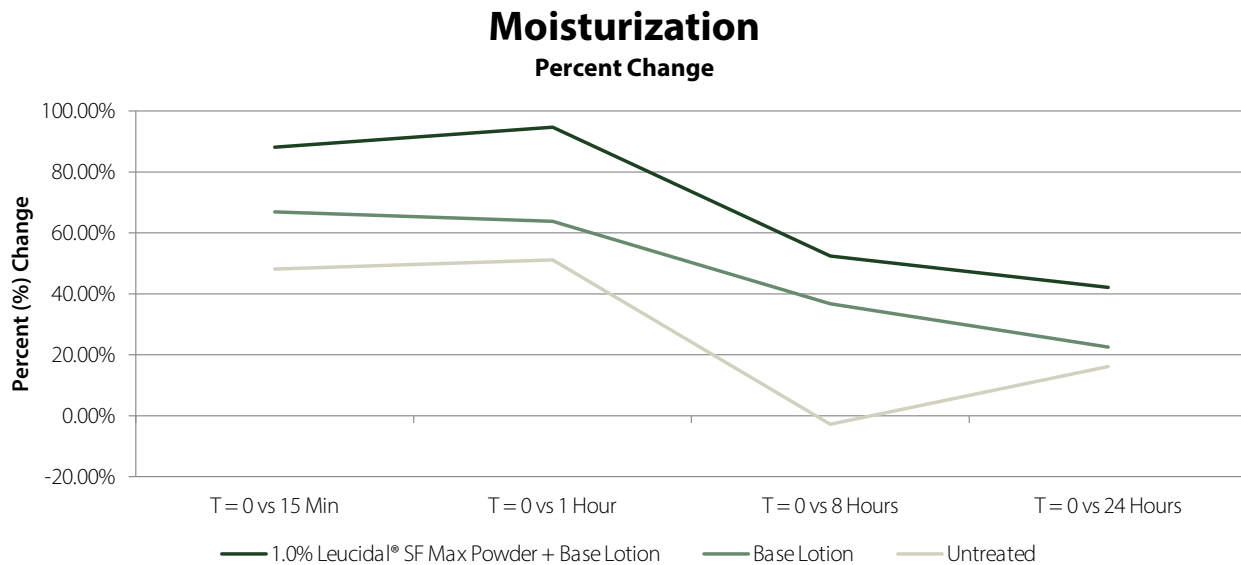


Figure 1. Percent Change in Moisturization.

Leucidal® SF Max Powder has the ability to more effectively inhibit the growth of a variety of bacteria and fungi than its liquid version, **Leucidal® SF Max**. The powder version is about five times more concentrated. Therefore, better results are achieved while also using less product.

Leucidal® SF Max Powder

US Patent Number 10,232,197

In Table 2, the Minimum Inhibitory Concentration (MIC) test shows comparative results between the two ingredients and shows that both materials provide broad-spectrum antimicrobial protection. The positive MIC screening results warranted further testing to confirm its ability to provide product preservation.

Organisms	Minimum Inhibitory Concentration (%)	
	Leucidal® SF Max (M15019MAX)	Leucidal® SF Max Powder (M15031)
<i>E. coli</i> #8739	0.50	0.12
<i>S. aureus</i> #6538	0.50	0.12
<i>P. aeruginosa</i> #9027	0.50	0.12
<i>C. albicans</i> #10231	0.50	0.12
<i>A. brasiliensis</i> #16404	0.50	0.12

Figure 2. MIC Results

Double Challenge Tests were completed using 1.0% **Leucidal® SF Max Powder** in the water phase of a generic base lotion formula. Samples were inoculated with *E. coli*, *P. aeruginosa*, *S. aureus*, *C. albicans*, and *A. brasiliensis*.

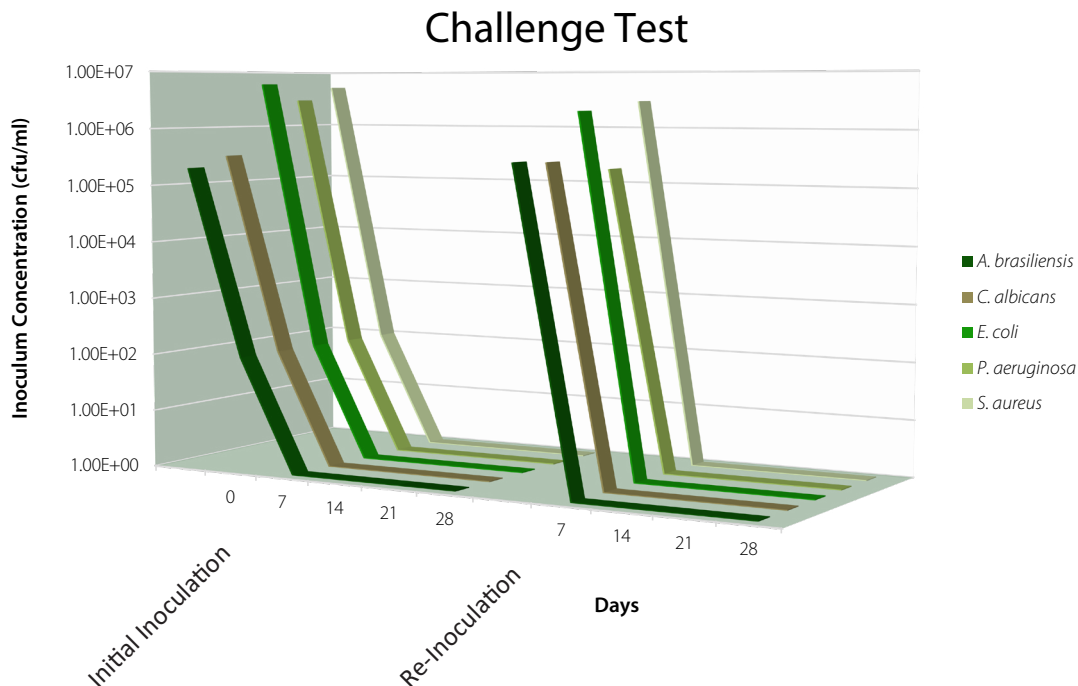


Figure 3. Challenge Test results for Generic Base Lotion Formula with 1.0% Leucidal® SF Max Powder inoculated on Day 0 and re-inoculated on Day 28. Results show log reduction in viable organisms.

Leucidal® SF Max Powder

US Patent Number 10,232,197

A Time Kill Test was performed to determine the change in population of aerobic microorganisms within a specified sampling time when tested against 1.0% **Leucidal® SF Max Powder** solution. The activity of the test material inoculated was evaluated at determined 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 after the inoculation.



Figure 4. Time Kill Test results for 1.0% **Leucidal® SF Max Powder**

USE RECOMMENDATIONS

Leucidal® SF Max Powder can be used in a wide range of cosmetic products; however, to ensure optimum results we recommend using the following guidelines. Incorporate the product into formulations at a pH between 3 and 7 during the aqueous phase of the process at temperatures lower than 70°C.

US Patent Number 10,232,197

Specification

Product Name: Leucidal® SF Max Powder
Code Number: M15031
CAS #'s: 1686112-36-6 (or) 68333-16-4
EINECS #'s: N/A (or) N/A
INCI Name: Lactobacillus Ferment

Specification	Parameter
Appearance	Free Flowing Powder*
Color	White to Pale Yellow
Odor	Characteristic
Loss on Drying (1g/1hr/105°C)	5.0% Maximum
pH (1% Solution in Water)	4.5 – 7.5
Heavy Metals	< 20 ppm
Lead	< 10 ppm
Arsenic	< 2 ppm
Cadmium	< 1 ppm
Bacteriocins (HPLC)	20.0 – 25.0%
Minimum Inhibitory Concentration ¹ Organism (ATCC#)	
E. coli (#8739)	0.12 – 0.25%
S. aureus (#6538)	0.05 – 0.25%
P. aeruginosa (#9027)	0.12 – 0.25%
C. albicans (#10231)	0.12 – 0.25%
A. brasiliensis (#16404)	0.12 – 0.25%

*HYGROSCOPIC

Note:

- 1) Refer to Inhibition Activity Data



Compositional Breakdown

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

Leucidal[®] SF Max Powder Code: M15031

Compositional Breakdown:

Ingredient	%
Lactobacillus Ferment	100.00

Information contained in this technical literature is believed to be accurate and is offered in good faith for the benefit of the customer. The company, however, cannot assume any liability or risk involved in the use of its chemical products since the conditions of use are beyond our control. Statements concerning the possible use of our products are not intended as recommendations to use our products in the infringement of any patent. We make no warranty of any kind, expressed or implied, other than that the material conforms to the applicable standard specification.



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

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Compositional Breakdown

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 (704) 276-7100 • Fax (704) 276-7101

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

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Inhibition Activity Data

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(704) 276-7100 • Fax (704) 276-7101

Product Name: Leucidal® SF Max & Leucidal® SF Max Powder
Code Number: M15019MAX & M15031
Test Request Number: 9456
CAS #'s: 1686112-36-6 (or) 68333-16-4
EINECS #'s: N/A (or) N/A
INCI Name: Lactobacillus Ferment

Organism (ATCC #)	Minimum Inhibitory Concentration (%)	
	Leucidal® SF Max M15019MAX	Leucidal® SF Max Powder M15031
<i>E.coli</i> #8739	0.50	0.12
<i>S. aureus</i> #6538	0.50	0.12
<i>P. aeruginosa</i> #9027	0.50	0.12
<i>C. albicans</i> #10231	0.50	0.12
<i>A. brasiliensis</i> #16404	0.50	0.12

QA Signature _____ Monica Beltran _____

Date _____ August 4, 2022 _____

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Antimicrobial Efficacy Test PCPC Section 20 Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

Leucidal® SF Max Powder

Test Request Form

7995

Purpose

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

Study Dates

The study was started on June 23rd, 2021 and was completed on August 27th, 2021.

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 with 0.5% of a solution of 20% Leucidal® SF Max Powder in water 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	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>A. brasiliensis</i>	<i>C. albicans</i>
	2.0×10^6	4.0×10^6	3.0×10^6	4.0×10^5	3.4×10^5
Day 0	99.716%	99.998%	99.829%	99.910%	99.991%
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%
Inoculum (re-inoculated) CFU/ml	2.0×10^6	1.2×10^5	5.0×10^6	5.0×10^6	3.0×10^5
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 Generic Cream Formula 0.5% of a solution of 20% Leucidal® SF Max Powder in water 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.

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 with 0.5% of a solution of 20% Leucidal® SF Max Powder in water. 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.

Phase	Ingredient	Supplier	%
I	Water	-	71.15%
	Carbopol Ultrez 10	Lubrizol	0.10%
II	Dermol 816	Alzo International	5.00%
	Sunflower Oil	Arista Industries	5.00%
	Silderm Emulsifying CS	Active Concepts, LLC	2.00%
	Dermol 2014	Alzo International	5.00%
	Lipomulse 165	Lipo Chemicals	1.60%
	Lipowax D	Lipo Chemicals	1.60%
	Stearic Acid	Acme Hardesty Oleochemicals	0.25%
III	ACB Bamboo Bioferment	Active Concepts, LLC	2.00%
IV	Organic Rice Solution	Arbor Organic Technologies, LLC	5.00%
	ACB Bio-Chelate 5	Active Concepts, LLC	2.00%

Manufacturing Process:

Phase I:

Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75°C. Slowly sift in Carbopol while mixing. Check pH and adjust to 6.0 – 7.0 with Citric Acid (50%) or NaOH (25%).

Phase II:

In a separate beaker, combine ingredients and heat to 75°C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75°C and mix for 15 minutes. Begin force cooling.

Phase III:

Add ingredients at 50°C and mix until homogenous.

Phase IV:

Add ingredients and mix until homogenous.

Specifications:

Appearance: White to Off-White Emulsion

pH: 4.0 – 5.5

*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.

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.

The information contained in this report is provided by Active Micro Technologies after the exercise of all reasonable care and skill in its compilation, preparation, and issue. It is provided without liability regarding its subsequent application and use. This type of screening test will be conducted only for validation of the efficacy of the antimicrobial agent or preservative system in the specific formulation tested. It does not address the suitability of the overall formula, nor does it address the regulatory status of any component therein. This testing does not account for the possibility of environmental microorganisms and cannot be relied upon as sufficient to justify commercialization of the product tested. By submitting samples for testing, the customer acknowledges that they will not hold Active Micro Technologies responsible for products launched based solely on the support of these studies.



Antimicrobial Efficacy Test PCPC Section 20 Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

Leucidal® SF Max Powder

Test Request Form

7996

Purpose

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

Study Dates

The study was started on June 23rd, 2021 and was completed on August 27th, 2021.

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.

Test Method

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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					
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	2.0×10^6	4.0×10^6	3.0×10^6	4.0×10^5	3.4×10^5
Day 0	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
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%
Inoculum (re-inoculated) CFU/ml	2.0×10^6	1.2×10^5	5.0×10^6	5.0×10^6	3.0×10^5
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 Generic Cream Formula 1.0% of a solution of 20% Leucidal® SF Max Powder in water on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

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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 with 1.0% of a solution of 20% Leucidal® SF Max Powder in water. 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.

Phase	Ingredient	Supplier	%
I	Water	-	71.15%
	Carbopol Ultrez 10	Lubrizol	0.10%
II	Dermol 816	Alzo International	5.00%
	Sunflower Oil	Arista Industries	5.00%
	Silderm Emulsifying CS	Active Concepts, LLC	2.00%
	Dermol 2014	Alzo International	5.00%
	Lipomulse 165	Lipo Chemicals	1.60%
	Lipowax D	Lipo Chemicals	1.60%
	Stearic Acid	Acme Hardesty Oleochemicals	0.25%
III	ACB Bamboo Bioferment	Active Concepts, LLC	2.00%
IV	Organic Rice Solution	Arbor Organic Technologies, LLC	5.00%
	ACB Bio-Chelate 5	Active Concepts, LLC	2.00%

Manufacturing Process:

Phase I:

Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75°C. Slowly sift in Carbopol while mixing. Check pH and adjust to 6.0 – 7.0 with Citric Acid (50%) or NaOH (25%).

Phase II:

In a separate beaker, combine ingredients and heat to 75°C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75°C and mix for 15 minutes. Begin force cooling.

Phase III:

Add ingredients at 50°C and mix until homogenous.

Phase IV:

Add ingredients and mix until homogenous.

Specifications:

Appearance: White to Off-White Emulsion

pH: 4.0 – 5.5

*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.

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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Antimicrobial Efficacy Test

PCPC Section 20

Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

Leucidal® SF Max Powder

Test Request Form

9524

Purpose

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

Study Dates

The study was started on September 9th, 2022 and was completed on November 11th, 2022.

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.

Test Method

Leucidal® SF Max Powder 0.5% was incorporated to the water phase of Generic Base Lotion Formula during manufacturing process. Fifty grams of this Generic Cream Formula 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	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>A. brasiliensis</i>	<i>C. albicans</i>
	6.0×10^6	3.0×10^6	5.2×10^6	2.0×10^5	3.1×10^5
Day 0	99.997%	>99.999%	99.983%	99.970%	99.999%
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%
Inoculum (re-inoculated) CFU/ml	2.1×10^6	2.0×10^5	3.0×10^6	3.2×10^5	3.0×10^5
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 Generic Base Lotion Formula with 0.5% Leucidal® SF Max Powder 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.

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 Base Lotion Formula with 0.5% Leucidal® SF Max Powder. 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.

Phase	Ingredient	Supplier	%
I	Water	-	85.2
	Carbopol Ultrez 10	Lubrizol	0.1
	Glycerin	PT. Musim Mas	3.0
II	Tealan	RITA	0.9
	Cetyl Alcohol	RITA	2.0
	Stearic Acid	Acme Hardesty Oleochemicals	0.8
	Glyceryl Stearate	Protameen Chem.	1.5
	Isopropyl Myristate	Alzo	1.5
	Mineral Oil	RITA	5.0

Manufacturing Process:

1. Phase I:

Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75°C. Slowly sift in Carbopol while mixing. Add the rest of ingredients.

2. Phase II:

In a separate beaker, combine ingredients and heat to 75°C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75°C and mix for 30 minutes. Begin force cooling to 25°C.

3. Check the pH and adjust.

Specifications:

Appearance: White to Off-White Emulsion

pH: 6.5 – 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.

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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Antimicrobial Efficacy Test

PCPC Section 20

Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

Leucidal® SF Max Powder

Test Request Form

9524

Purpose

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

Study Dates

The study was started on September 9th, 2022 and was completed on November 11th, 2022.

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.

Test Method

Leucidal® SF Max Powder 1.0% was incorporated to the water phase of Generic Base Lotion Formula during manufacturing process. Fifty grams of this Generic Cream Formula 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	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>A. brasiliensis</i>	<i>C. albicans</i>
	6.0×10^6	3.0×10^6	5.2×10^6	2.0×10^5	3.1×10^5
Day 0	99.993%	>99.999%	99.980%	99.950%	99.967%
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%
Inoculum (re-inoculated) CFU/ml	2.1×10^6	2.0×10^5	3.0×10^6	3.2×10^5	3.0×10^5
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 Generic Base Lotion Formula with 1.0% Leucidal® SF Max Powder in water 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.

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 Base Lotion Formula with 1.0% Leucidal® SF Max Powder in water. 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.

Phase	Ingredient	Supplier	%
I	Water	-	85.2
	Carbopol Ultrez 10	Lubrizol	0.1
	Glycerin	PT. Musim Mas	3.0
II	Tealan	RITA	0.9
	Cetyl Alcohol	RITA	2.0
	Stearic Acid	Acme Hardesty Oleochemicals	0.8
	Glyceryl Stearate	Protameen Chem.	1.5
	Isopropyl Myristate	Alzo	1.5
	Mineral Oil	RITA	5.0

Manufacturing Process:

1. Phase I:

Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75°C. Slowly sift in Carbopol while mixing. Add the rest of ingredients.

2. Phase II:

In a separate beaker, combine ingredients and heat to 75°C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75°C and mix for 30 minutes. Begin force cooling to 25°C.

3. Check the pH and adjust.

Specifications:

Appearance: White to Off-White Emulsion

pH: 6.5 – 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.

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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(704) 276-7100 • Fax (704) 276-7101

Time Kill Test E2315 Assessment of Antimicrobial Activity Using a Time Kill Procedure

Product

Leucidal® SF Max Powder

Test Request #:

9501

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 August 24th, 2022 and was completed on August 29th, 2022.

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:

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.

Information contained in this technical literature is believed to be accurate and is offered in good faith for the benefit of the customer. The company, however, cannot assume any liability or risk involved in the use of its chemical products since the conditions of use are beyond our control. Statements concerning the possible use of our products are not intended as recommendations to use our products in the infringement of any patent. We make no warranty of any kind, expressed or implied, other than that the material conforms to the applicable standard specification.

Test Method

Leucidal® SF Max Powder was reconstituted at 20% in distilled water, and ten grams of 0.5% from this solution 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 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 5 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
<i>E.coli</i> * ATCC# 8739	1.4×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
<i>S.aureus</i> ATCC# 6538	5.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
<i>P.aeruginosa</i> ATCC# 9027	3.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
<i>A.brasiliensis</i> ATCC# 16404	2.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
<i>C.albicans</i> ATCC# 10231	2.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%

Table 1. Time Kill Test results for 0.5% Leucidal® SF Max Powder Solution 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.

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 0.5% Leucidal® SF Max Powder 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.

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Time Kill Test E2315 Assessment of Antimicrobial Activity Using a Time Kill Procedure

Product

Leucidal® SF Max Powder

Test Request #:

9502

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 August 24th, 2022 and was completed on August 29th, 2022.

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:

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

Leucidal® SF Max Powder was reconstituted at 20% in distilled water, and ten grams of 1.0% from this solution 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 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 5 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
<i>E.coli</i> * ATCC# 8739	1.4×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
<i>S.aureus</i> ATCC# 6538	5.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
<i>P.aeruginosa</i> ATCC# 9027	3.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
<i>A.brasiliensis</i> ATCC# 16404	2.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
<i>C.albicans</i> ATCC# 10231	2.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%

Table 1. Time Kill Test results for 1.0% Leucidal® SF Max Powder Solution 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.

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 1.0% Leucidal® SF Max Powder 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.



Moisturization Assay

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

Tradename: Leucidal SF Max Powder

Code: M15031

CAS #: 1686112-36-6 (or) 68333-16-4

Test Request Form #: 9474

Lot #: N210329B

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*

Study Director: *Maureen Danaher*

Principle Investigator: *Grant Tyler*

Test Performed:

Moisturization Assay

Introduction

An *in-vivo* study was conducted over a period of 24 hours to evaluate the moisturization benefits of **Leucidal SF Max Powder**. 20 M/F subjects between the ages of 23-45 participated in the study. Results indicate that **Leucidal SF Max Powder** is capable of significantly increasing moisturization.

The Moisturization Assay was conducted to assess the moisturizing ability of **Leucidal SF Max Powder**.

Materials

A. Equipment: DermaLab Skin Combo (Hydration/ Moisture Pin Probe)

Methods

The moisture module provides information about the skin's hydration by measuring the conducting properties of the upper skin layers when subjected to an alternating voltage. The method is referred to as a conductance measurement and the output is presented in the unit of uSiemens (uS). A moisture pin probe is the tool used to gather hydration values.

20 volunteers M/F between the ages of 23 and 45 and who were known to be free of any skin pathologies participated in this study. A Dermalab Corneometer was used to measure the moisture levels on the subject's volar forearms. The Corneometer is an instrument that measures the amount of water within the skin. The presence of moisture in the skin improves conductance therefore results in higher readings than dry skin. Therefore the higher the levels of moisture, the higher the readings from the Corneometer will be. Baseline moisturization readings were taken on the first day of the study.



Moisturization Assay

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Following initial measurements, subjects were instructed to apply 2 mg of each treatment to their volar forearm. Measurements were taken at intervals of 15 minutes, 1 hour, 8 hours and 24 hours after the application of test materials.

For added perspective, measurements of an untreated test site and a site treated with a base lotion (Cetaphil Moisturizing for All Skin Types) were recorded.

Results

Leucidal SF Max Powder showed high moisturizing capabilities alone at both concentrations of 0.5% and 1.0%. Please note, each value is an average of three consecutive readings per test site.

Percent change in moisturization is calculated by the following formula:

$$\text{Percent (\%) Change} = \frac{\text{Average Moisture Value}_{T=24 \text{ hours.etc}} - \text{Average Baseline Value}_{T=0}}{\text{Average Baseline Value}_{T=0}} \times 100$$

Averages	T = 0	T = 15 Minutes	T = 1 Hour	T = 8 Hours	T = 24 Hours
Experimental (1.0% Leucidal SF Max Powder + Base Lotion)	129.45	243.50	252.00	197.30	184.00
Experimental (0.5% Leucidal SF Max Powder + Base Lotion)	125.75	230.50	228.25	187.00	168.15
Base Lotion	130.47	217.74	213.74	178.37	159.89
Untreated	128.30	190.10	193.90	124.70	149.00

Table 1. Average Moisture Increase of Individual Test Sites.

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Percent (%) Change	T = 0 vs 15 Minutes	T = 0 vs 1 Hour	T = 0 vs 8 Hours	T = 0 vs 24 Hours
Experimental (1.0% Leucidal SF Max Powder + Base Lotion)	88.10	94.67	52.41	42.14
Experimental (0.5% Leucidal SF Max Powder + Base Lotion)	83.30	81.51	48.71	33.72
Base Lotion	66.88	63.82	36.71	22.55
Untreated	48.17	51.13	-2.81	16.13

Table 2. Comparative Moisture Increase from Baseline.

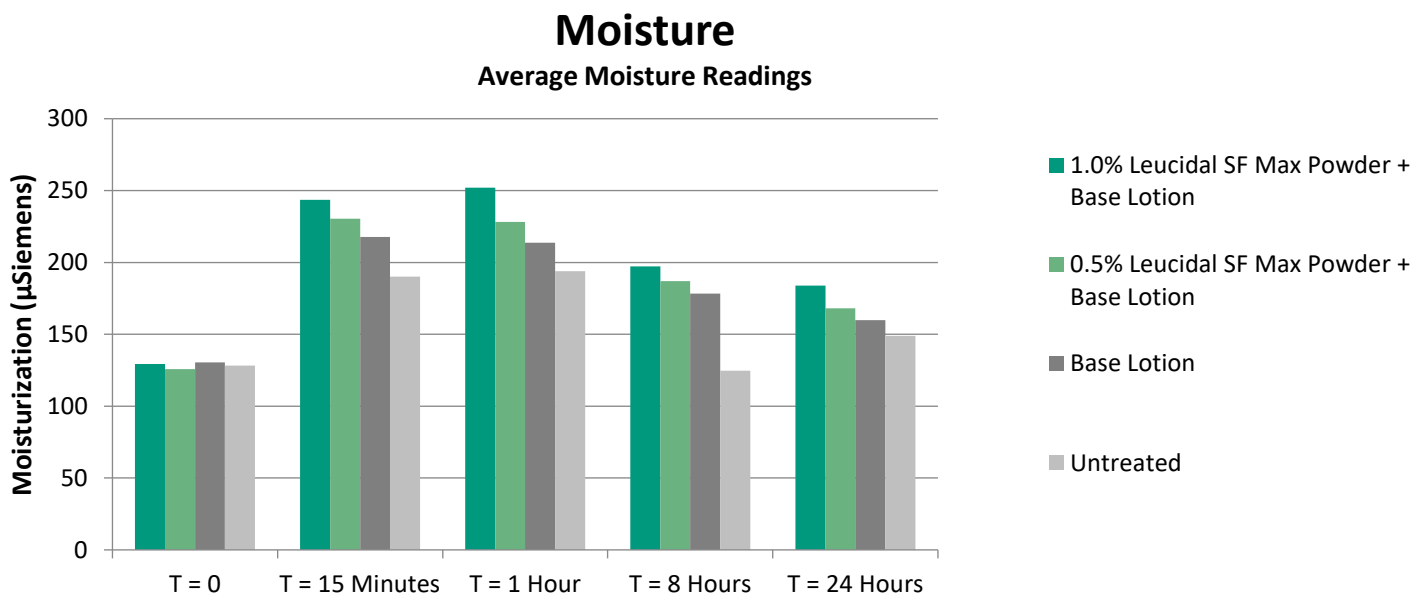


Figure 1. Average Increase in Moisturization

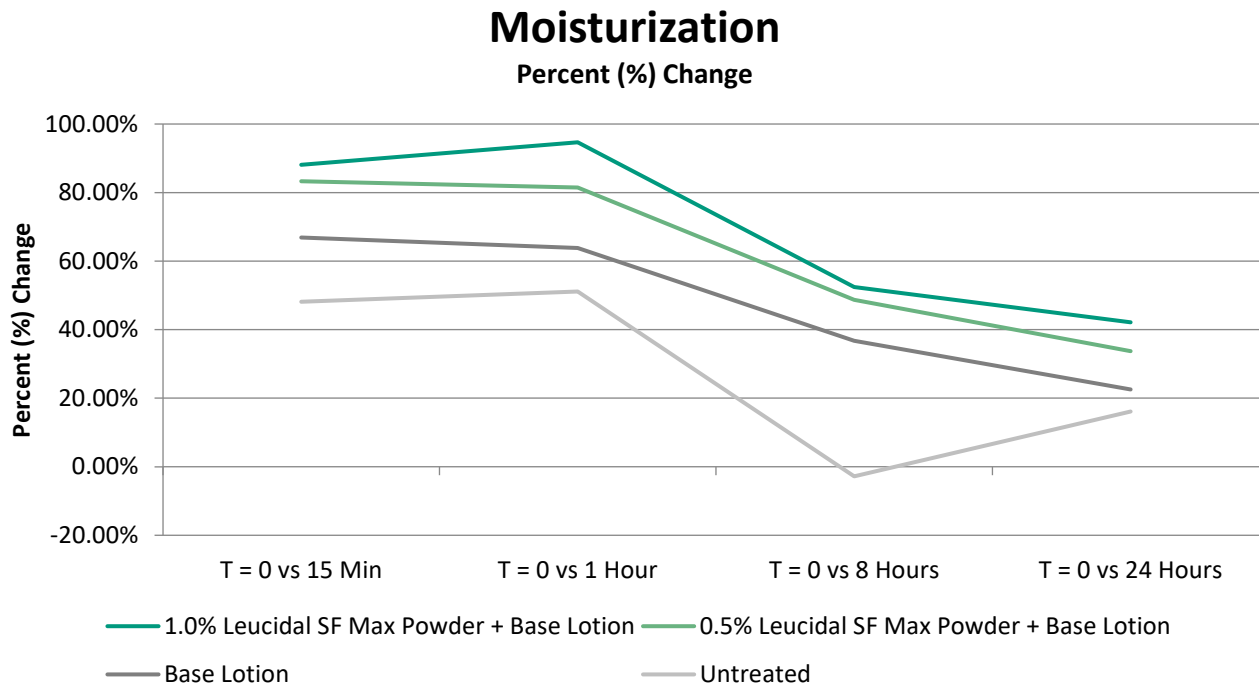


Figure 2. Percent Change in Moisturization of Each Time Point Compared to Baseline

Discussion

Leucidal SF Max Powder in a base lotion demonstrated the ability to enhance moisturization to a greater degree than the base lotion alone at concentrations of 0.5% and 1.0%.

As evidenced in a 24 hour study of 0.5% **Leucidal SF Max Powder** on skin, moisture levels were improved by 83.30% after 15 minutes and 33.72% after 24 hours as compared to baseline values. At a use level of 1.0% in a base lotion, moisture levels improved by 88.10% after 15 minutes and 42.14% after 24 hours.

With the present study, we can confirm that **Leucidal SF Max Powder** is capable of providing moisturizing and hydrating benefits when added to personal care applications.



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

Date Issued: May 12, 2023

ALLERGEN DECLARATION

RE: *Leucidal® SF Max Powder (M15031)*

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.)

Molluscs – or molluscs products

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

Information contained in this technical literature is believed to be accurate and is offered in good faith for the benefit of the customer. The company, however, cannot assume any liability or risk involved in the use of its chemical products since the conditions of use are beyond our control. Statements concerning the possible use of our products are not intended as recommendations to use our products in the infringement of any patent. We make no warranty of any kind, expressed or implied, other than that the material conforms to the applicable standard specification.

Test Article: Leucidal SF Max Powder
Code Number: M15031
CAS #: 1686112-36-6 (or) 68333-16-4

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

Study Director: Maureen Danaher
Principle Investigator: Monica Beltran

Test Performed:
Genotoxicity: Bacterial Reverse Mutation Test

Reference:
OECD471/ISO10993.Part3

Test Request Number: 10322

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 SF Max Powder** would cause mutagenic changes in the average number of revertants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535 and tryptophan-dependent *Escherichia coli* strain WP2uvrA 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 WP2uvrA after Sport Inhibition Screen.

Separate tubes containing 2 ml of molten top agar at 45°C 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°C. 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 WP2uvrA. 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 *uvrB* gene. Cultures of tester strains TA98 and TA100 demonstrated the presence of the Pkm101 plasmid R-factor. All WP2 *uvrA* cultures demonstrated the deletion in the *uvrA* 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, WP2uvrA, 10-60.

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* WP2uvrA 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 *uvrB* gene and the deletion in the *uvrA* gene that increase their ability to detect mutagens, respectively. These genetically altered *Salmonella typhimurium* strains (TA98, TA100, TA1537 and TA1535) and *Escherichia coli* strain (WP2uvrA) 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.

<u>Tester strain</u>	<u>Mutations/Genotypic Relevance</u>
TA98	hisD3052, Dgal chlD bio <i>uvrB rfa</i> pKM101
TA100	hisG46, Dgal chlD BIO <i>uvrB rfa</i> pKM101
TA1537	hisC3076, <i>rfa</i> , Dgal chlD bio <i>uvrB</i>
TA 1535	hisG46, Dgal chlD bio <i>uvrB rfa</i>
WP2uvrA	trpE, <i>uvrA</i>

<i>rfa</i>	=	causes partial loss of the lip polysaccharide wall which increases permeability of the cell to large molecules.
<i>uvrB</i>	=	deficient DNA excision-repair system (i.e., ultraviolet sensitivity)
pKM101	=	plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens.
<i>uvrA</i>	=	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.

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 revertant 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 2×10^9 /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 μ l of culture for each strain and 100 μ l of testing solution or vehicle without test material. A 500 μ l 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 50 μ l 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 revertants was determined. The mean numbers of revertants of the test plates were compared to the mean number of revertants 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 *uvrB* gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvrA* cultures must demonstrate the deletion in the *uvrA* 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*uvrA*, 10-60. To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to 0.3×10^9 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-toxic dose 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.

B.

C. 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.

D. Titer (Organisms/ml):

5 x 10⁸ UFC/ml plate count indicates that the initial population was in the range of 1 to 2 x 10⁹ 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*uvrA* 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 *uvrB* gene. Cultures of tester strains TA98 and TA100 demonstrated the presence of the Pkm101 plasmid R-factor. All WP2 *uvrA* cultures demonstrated the deletion in the *uvrA* 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*uvrA*, 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*uvrA*. 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.

Appendix 2:

Bacterial Mutation Assay Plate Incorporation Assay Results

	Concentration µg per Plate	TA98		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	29	35	32
	1500	19	17	18
	500	21	23	22
	150	18	30	24
	50	31	22	27
	15	18	20	19
	5.0	22	21	22
	1.5	19	19	19
Test Solution w/o S9	5000	20	22	21
	1500	35	32	34
	500	17	19	18
	150	20	20	20
	50	20	23	22
	15	21	21	21
	5.0	24	21	23
	1.5	22	18	20
DI Water w/S9		34	18	20
DI Water w/o S9		4	16	10
2-aminoanthracen w/ S9		365	387	376
2-nitrofluorene w/o S9		295	211	253
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

	Concentration µg per Plate	TA100		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	144	60	102
	1500	124	144	134
	500	224	163	180
	150	236	240	238
	50	180	168	174
	15	160	188	174
	5.0	148	300	224
	1.5	172	136	154
Test Solution w/o S9	5000	112	125	119
	1500	100	103	102
	500	135	140	138
	150	80	48	64
	50	92	108	100
	15	132	160	146
	5.0	108	192	150
	1.5	148	176	162
DI Water w/S9		168	144	156
DI Water w/o S9		180	46	113
2-aminoanthracen w/ S9		450	437	444
Sodium azide w/o S9		520	408	464
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

	Concentration µg per Plate	TA1537		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	11	9	10
	1500	13	12	13
	500	7	14	10
	150	11	6	9
	50	10	4	7
	15	13	8	11
	5.0	8	14	11
	1.5	13	8	11
Test Solution w/o S9	5000	30	20	25
	1500	19	10	15
	500	10	8	9
	150	4	8	6
	50	24	19	22
	15	10	12	11
	5.0	18	12	15
	1.5	16	10	13
DI Water w/S9		9	3	6
DI Water w/o S9		13	16	15
2-aminoanthracen w/ S9		314	312	313
2-aminoacridine w/o S9		320	304	312
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

	Concentration µg per Plate	TA1535		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	24	21	22
	1500	13	7	10
	500	28	21	25
	150	20	15	16
	50	22	20	21
	15	24	11	18
	5.0	22	32	27
	1.5	24	13	19
Test Solution w/o S9	5000	88	82	85
	1500	72	95	84
	500	81	80	81
	150	84	80	81
	50	12	8	10
	15	23	21	22
	5.0	8	18	13
	1.5	21	16	19
DI Water w/S9		18	15	17
DI Water w/o S9		18	30	24
2-aminoanthracen w/ S9		228	217	223
Sodium azide w/o S9		408	480	444
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

	Concentration µg per Plate	WP2uvrA		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	12	12	12
	1500	13	39	26
	500	43	40	42
	150	56	25	41
	50	38	48	43
	15	49	44	47
	5.0	57	55	56
	1.5	50	65	58
Test Solution w/o S9	5000	81	65	73
	1500	40	51	46
	500	25	32	29
	150	29	16	23
	50	33	30	32
	15	40	40	40
	5.0	50	44	47
	1.5	41	54	48
DI Water w/S9		48	41	45
DI Water w/o S9		50	51	51
2-aminoanthracen w/ S9		501	522	512
Methylmethanesulfonate w/o S9		360	230	300
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



Cellular Viability Assay Analysis

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

Tradename: Leucidal® SF Max Powder

Code: M15031

CAS #: 1686112-36-6 (or) 68333-16-4

Test Request Form #: 10258

Lot #: 9394433

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

Study Director: *Maureen Drumwright*

Principle Investigator: *Hannah Duckett*

Test Performed:

Cellular Viability Assay

Introduction

The cellular viability assay is useful for quantitatively measuring cell-mediated cytotoxicity, cell proliferation and mitochondrial metabolic activity. Increased metabolism in a cell indicates ample cellular respiration and adenosine triphosphate (ATP) production. ATP is the molecular energy of cells and is required in basic cell function and signal transduction. A decrease in ATP levels indicates cytotoxicity and decreased cell function while an increase in ATP levels indicates healthy cells.

The cellular viability assay was conducted to assess the ability of **Leucidal® SF Max Powder** to increase cellular metabolic activity in cultured dermal fibroblasts.

Assay Principle

The assay utilizes a nonfluorescent dye, resazurin, which is converted to a fluorescent dye, resorufin, in response to chemical reduction of growth medium from cell growth and by respiring mitochondria. Healthy cells that are in a proliferative state will be able to easily convert resazurin into resorufin without harming the cells. This method is a more sensitive assay than other commonly used mitochondrial reductase dyes such as MTT. An increase in the signal generated by resazurin-conversion is indicative of a proliferative cellular state.

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Materials

- A. Kit:** PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261)
B. Incubation Conditions: 37°C at 5% CO₂ and 95% relative humidity (RH)
C. Equipment: Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Pipettes
D. Cell Line: Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
E. Media/Buffers: Basal Medium (Fibrolife; LM-0001), 500µg/mL Human Serum Albumins (Fibrolife; LS-1001), 0.6µM Linoleic Acid (Fibrolife; LS-1001), 0.6µg/mL (Fibrolife; LS-1001), 5ng/mL Fibroblast Growth Factor (Fibrolife; LS-1002), 5mg/mL Epidermal Growth Factor (Fibrolife; LS-1003), 30pg/mL Transforming Growth Factor β-1 (Fibrolife; LS-2003), 7.5mM L-Glutamine (Fibrolife; LS-1006), 1µg/mL Hydrocortisone Hemisuccinate (Fibrolife; LS-1007), 50µg/mL Ascorbic Acid (Fibrolife; LS-1005), 5µg/mL Insulin (Fibrolife; LS-1004)
F. Culture Plate: Falcon flat bottom 96-well tissue culture treated plates
G. Reagents: PrestoBlue™ reagent (10X)
H. Other: Sterile disposable pipette tips

Methods

Human dermal fibroblasts were seeded into 96-well tissue culture plates and allowed to grow to confluency in complete serum-free media. A 10-fold serial dilution was performed resulting in **Leucidal® SF Max Powder** concentrations of 0.01%, and 0.1% in complete serum-free media and incubated with fibroblasts for 24 hours.

Ten microliters of viability reagent was added to 90µL of cell culture media in culture wells and a fluorometric measurement was taken at 560nm for excitation and 590nm for emission.

Results

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

Leucidal® SF Max Powder did not exhibit negative effects on cell metabolism.

Cellular metabolism results are shown as mean fluorescence units (MFU) and expressed as percentage change, calculated by the below equation:

$$\text{Percent (\%) Change} = \frac{MFU_{\text{Sample}} - MFU_{\text{Control}}}{MFU_{\text{Control}}} \times 100$$

Cellular Viability Leucidal® SF Max Powder

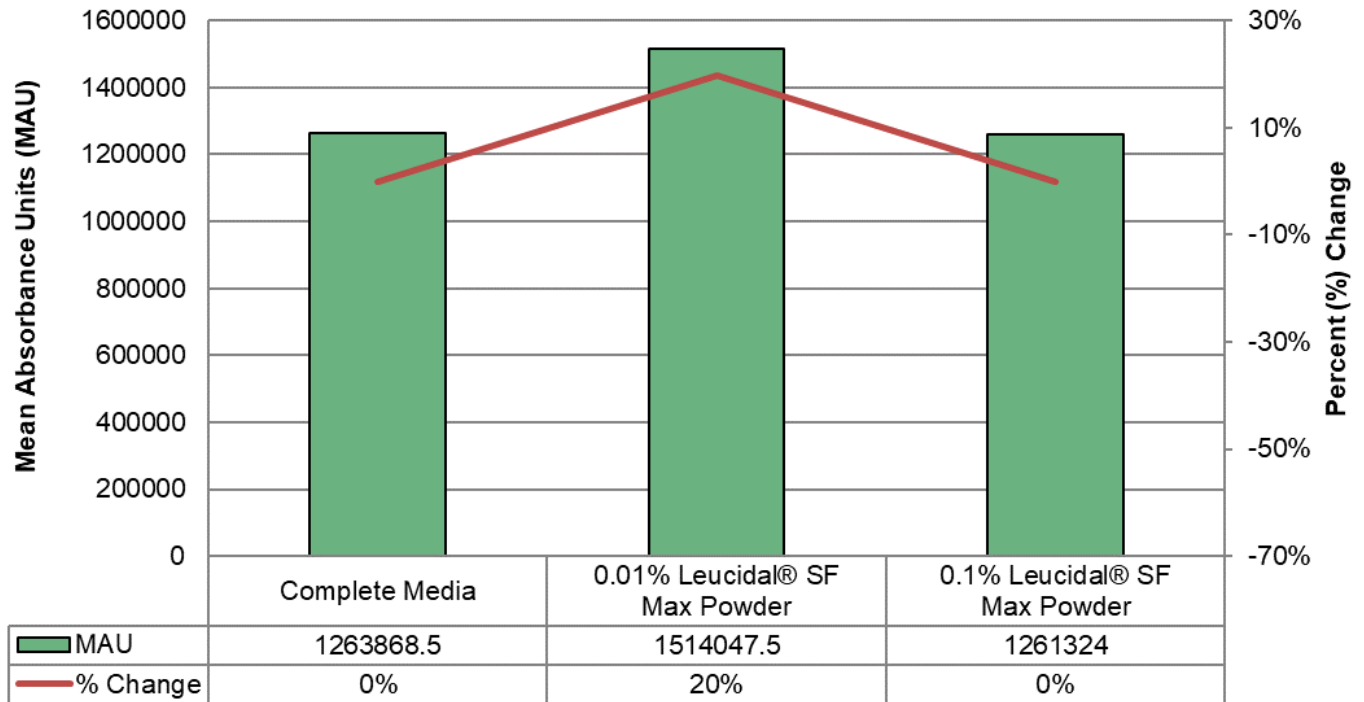


Figure 1: Cellular Metabolism of Leucidal® SF Max Powder-Treated Fibroblasts

Discussion

In this study, **Leucidal® SF Max Powder (M15031)** was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At concentrations of 0.01%, and 0.1%, **Leucidal® SF Max Powder**, nor the preservatives contained therein exhibited any inhibition of cell viability. It can therefore be concluded that at normal use concentrations **Leucidal® SF Max Powder** is not cytotoxic.



Dermal and Ocular Irritation Tests

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

Tradename: Leucidal® SF Max Powder

Code: M15031

CAS #: 1686112-36-6 (or) 68333-16-4

Test Request Form #: 10287

Lot #: 841500

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

Study Director: Maureen Drumwright

Principle Investigator: Daniel Shill

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® SF Max Powder** 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 a **non-irritant**. 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% CO₂, 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-irritating**. The negative and positive controls performed as anticipated.

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.

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Dermal and Ocular Irritation Tests

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

II. Materials

- A. Incubation Conditions:** 37°C at 5% CO₂ 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 H₂O
- 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%.

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% CO₂ 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% CO₂ 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% CO₂, 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% CO₂, 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% CO₂, 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 (OD₅₇₀) 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.

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 Figures 1 and 2. In no case was the tissue viability $\leq 50\%$ for EpiDerm™ or $\leq 60\%$ 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.

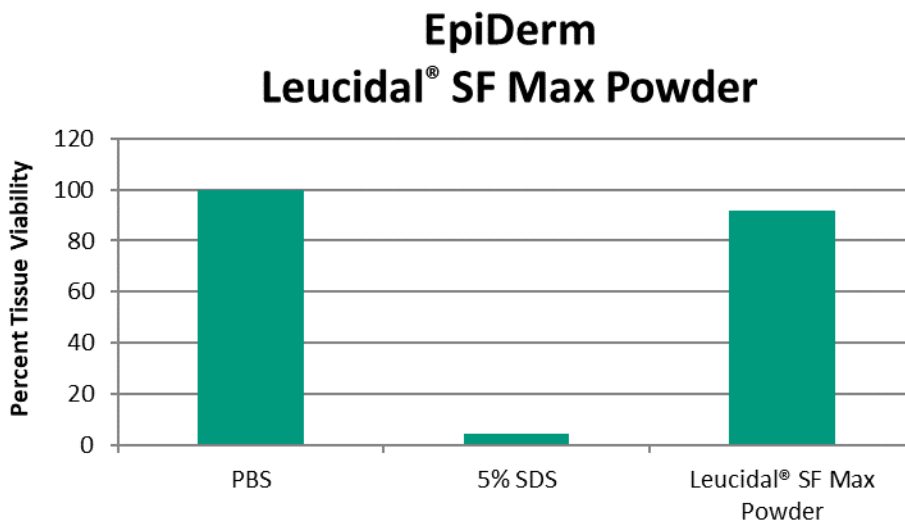


Figure 1: EpiDerm tissue viability

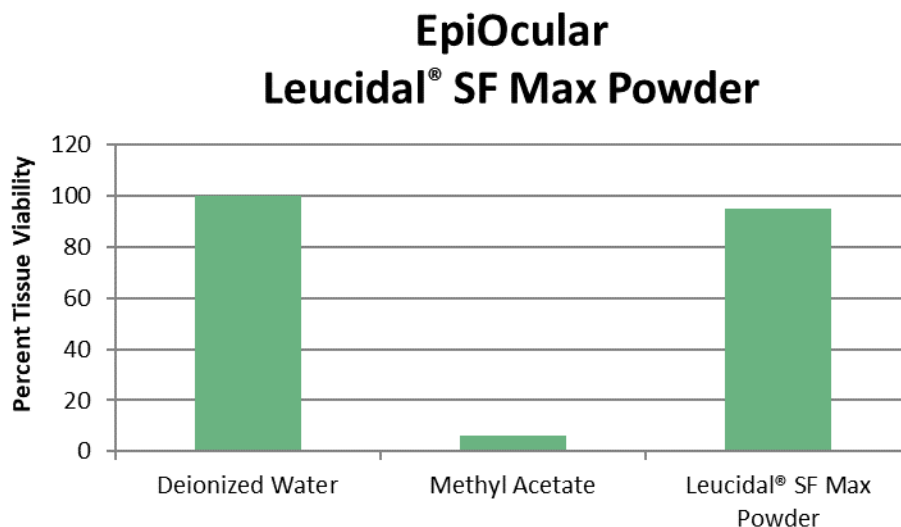


Figure 2: EpiOcular tissue viability

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OECD 201 Freshwater Alga Growth Inhibition Test

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

Tradename: Leucidal® SF Max Powder

Code: M15031

CAS #: 1686112-36-6 (or) 68333-16-4

Test Request Form #: 10191

Lot #: 841500

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® SF Max Powder** 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 EC₁₀ and EC₂₀ 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 EC₅₀ 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 EC₅₀ 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

- Inoculum 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* $5 \times 10^{3-4}$ 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 $E_r C_x$
- 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.

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 EC₁₀ and EC₂₀.

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 EC₁₀ and EC₂₀ 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 EC₁₀ and EC₂₀.

$$\text{Percent (\%) Inhibition} = \frac{\mu_c - \mu_T}{\mu_c} \times 100$$

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

Results

General Information:

Name of new chemical substance	Leucidal® SF Max Powder		
INCI Nomenclature	Lactobacillus Ferment		
CAS number	1686112-36-6 (or) 68333-16-4		
Formulation Method	Fermentation		
Molecular weight	4800 Da		
Purity of the new chemical substance used for the test (%)	100%		
Lot number of the new chemical substance used for the test	841500		
Names and contents of impurities	n/a		
Solubility in water	Soluble		
Properties at room temperature	Free Flowing White to Pale Yellow Powder, Characteristic Odor		
Stability	Stable Under Normal Conditions		
Solubility in solvents, etc.	Solvent	Solubility	Stability in solvent
	N/A	N/A	N/A

Test Materials and Methods:

Items		Contents	
Test Organisms	Species	<i>Pseudokirchneriella subcapitata</i>	
	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	04/03/2023	
	Test Concentrations	200, 89.4, 42.3, 19.2, 7.8 mg/L	
	Number of organisms	5 x 10 ³⁻⁴ 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	139.24 mg/L and 258.81 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® SF Max Powder** was determined to be 139.24 mg/L EC₁₀ and 258.81 mg/L EC₂₀. 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.

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OECD 301B Ready Biodegradability Assay

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

Tradename: Leucidal® SF Max Powder

Code: M15031

CAS #: 1686112-36-6 (or) 68333-16-4

Test Request Form #: 10148

Lot #: 841500

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

Study Director: *Maureen Drumwright*

Principle Investigator: *Daniel Shill*

Test Performed:

OECD 301 B

Ready Biodegradability: CO₂ Evolution (Modified Sturm Test)

Introduction

A study was conducted to assess the ready biodegradability of **Leucidal® SF Max Powder** in an aerobic aqueous medium. In the OECD guideline 301 for ready biodegradability, six methods are provided as options. This report uses method B, CO₂ 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.



OECD 301B Ready Biodegradability Assay

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The pass levels for ready biodegradability are 70% removal of DOC and 60% of ThOD (Theoretical Oxygen Demand) or ThCO₂ (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 CO₂ 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 ThCO₂ 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, KH₂PO₄.....8.5g
 - Dipotassium hydrogen orthophosphate, K₂HPO₄.....21.8g
 - Disodium hydrogen orthophosphate dehydrate, Na₂HPO₄·2H₂O.....33.4g
 - Ammonium chloride, NH₄Cl.....0.5g
 - Solution B (Dissolve in water and make up to 1 liter)
 - Calcium chloride, anhydrous, CaCl₂.....27.50g
 - Or
 - Calcium chloride dehydrate, CaCl₂·2H₂O.....36.40g
 - Solution C (Dissolve in water and make up to 1 liter)
 - Magnesium sulphate heptahydrate, MgSO₄·7H₂O.....22.50g
 - Solution D (Dissolve in water and make up to 1 liter.)
 - Iron (III) chloride hexahydrate, FeCl₃·6H₂O.....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 CO₂-free oxygen and CO₂-free nitrogen from gas cylinders in the correct proportions (20% O₂ : 80% N₂)
 - 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, making sure it
- 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 CO₂-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 CO₂-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)

- II. Start the test by bubbling CO₂-free air through the suspensions at a rate of 30-100 mL/minute.
- III. CO₂ Determination
- It is mandatory to follow the CO₂ evolution from the test suspensions and inoculum blanks in parallel and it is advisable to do the same for the other test vessels.
 - During the first ten days it is recommended that analyses of CO₂ 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 CO₂ 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.
 - 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
- Data from the test should be entered onto the attached data sheet.
 - The amount of CO₂ 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.
 - Since 1 mmol of CO₂ is produced for every mol of Ba(OH)₂ reacted to BaCl₂ and 2 mmol of HCl are needed for the titration of the remaining Ba(OH)₂ and given that the molecular weight of CO₂ is 44 g, the weight of CO₂ 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 CO₂ produced is 1.1 in this case. Calculate the weights of CO₂ produced from the inoculum alone and from the inoculum plus test substance using the respective titration values. The difference is the weight of CO₂ produced from the test substance alone.

d. The percentage biodegradation is calculated from:

$$\% \text{ Degradation} = \frac{\text{mg CO}_2 \text{ Produced}}{\text{ThCO}_2 \times \text{mg Test Substance Added}} \times 100$$

Or

$$\% \text{ Degradation} = \frac{\text{mg CO}_2 \text{ Produced}}{\text{mg TOC Added in Test} \times 3.67} \times 100$$

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 CO₂ produced after any time interval from the concentration of inorganic carbon and the volume of absorbent used. Calculate the percentage degradation from:

$$\% \text{ ThCO}_2 = \frac{\text{mg IC from Test Flask} - \text{mg IC from Blank}}{\text{mg TOC Added as Test Substances}} \times 100$$

- 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:

$$\% \text{ Abiotic Degradation} = \frac{\text{CO}_2 \text{ Produced by Sterile Flask After 28 Days (mg)}}{\text{ThCO}_2 \text{ (mg)}} \times 100$$

Validity of Tests

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 CO₂ 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 CO₂/L are obtained, the data and experimental technique should be examined critically.

Data Sheet

Laboratory	Active Concepts Tissue Culture Laboratory		
Test Start Date	03/13/2023		
Test Substance	Name	Leucidal® SF Max Powder	
	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
CO₂ Production and Degradability	Method	Ba(OH)₂	0.0125M
		NaOH	N/A
		Other	N/A
Total Contact Time	28 Days		
Total CO₂ Evolved Measurements	Days	2, 4, 11, 17, 23, 28	
Degradation Over Time	91.6% and 93.2% after 28 days		
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 test method, **Leucidal® SF Max Powder** achieved 92.4% biodegradation after 28 days of testing. The product met method requirements for the Readily Biodegradable classification.



OECD TG 442C: In Chemico Skin Sensitization

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(704) 276-7100 • Fax (704) 276-7101

Tradename: Leucidal® SF Max Powder

Code: M15031

CAS #: 1686112-36-6 (or) 68333-16-4

Test Request Form #: 10288

Lot #: 841500

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

Study Director: Maureen Drumwright

Principle Investigator: Daniel Shill

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 contact¹. 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 dermatitis². 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® SF Max Powder** 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.

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-RFAACAA-COOH); Lysine peptide (Ac-RFAAKAA-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® SF Max Powder** in Acetonitrile

*For mixtures and multi-constituent substances of known composition such as **Leucidal® SF Max Powder**, a single purity should be determined by the sum of the proportion of its constituents (excluding water), and a single apparent molecular weight determined by considering the individual molecular weights of each component in the mixture (excluding water) and their individual proportions. The resulting purity and apparent molecular weight can then be used to calculate the weight of test chemical necessary to prepare a 100 mM solution.

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
<ul style="list-style-type: none"> • 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) 	<ul style="list-style-type: none"> • 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)

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 ± 0.05 mM 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 ± 0.05 mM.

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

Therefore the measured values of % depletion in the three separated runs for each peptide depletion assay include:

Cysteine 1:10/Lysine 1:50 Prediction Model		
Mean of Cysteine and Lysine % Depletion	Reactivity Class	Prediction
3.09	Minimal Reactivity	Non-sensitizer
3.13	Minimal Reactivity	Non-sensitizer
3.14	Minimal Reactivity	Non-sensitizer

Cysteine 1:10 Prediction Model		
Mean of Cysteine and Lysine % Depletion	Reactivity Class	Prediction
2.99	Minimal Reactivity	Non-sensitizer
3.03	Minimal Reactivity	Non-sensitizer
3.02	Minimal Reactivity	Non-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:

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

 Based on HPLC-UV analysis of **Leucidal® SF Max Powder (M15031)** we can determine this product is not classified as a sensitizer and is not predicted to cause allergic contact dermatitis. The Mean Percent Depletion of Cysteine and Lysine was 3.07% causing minimal reactivity in the assay giving us the prediction of a non-sensitizer.



OECD TG 442D: *In Vitro* Skin Sensitization

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

Tradename: Leucidal® SF Max Powder

Code: M15031

CAS #: 1686112-36-6 (or) 68333-16-4

Test Request Form #: 10289

Lot #: 841500

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

Study Director: *Maureen Drumwright*

Principle Investigator: *Daniel Shill*

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 Chemicals¹. 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® SF Max Powder** 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



OECD TG 442D: In Vitro Skin Sensitization

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Materials

- A. **Incubation Conditions:** 37°C at 5% CO₂ 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® SF Max Powder** 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% CO₂ 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% CO₂. 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 IC₅₀ and IC₃₀ 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 EC_{1.5} 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.

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

1. The I_{max} 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 EC_{1.5} determining concentration)
3. The EC_{1.5} value is less than 1000 μM (or < 200 $\mu\text{g/ml}$ 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	31.38
DMSO	Non-Sensitizer	No Induction	243.24 μM	0.19
Leucidal® SF Max Powder	Non-Sensitizer	No Induction	> 1000 μM	0.38

Table 1: Overview of KeratinoSens™ Assay Results

**KeratinoSens™ Assay
Leucidal® SF Max Powder**

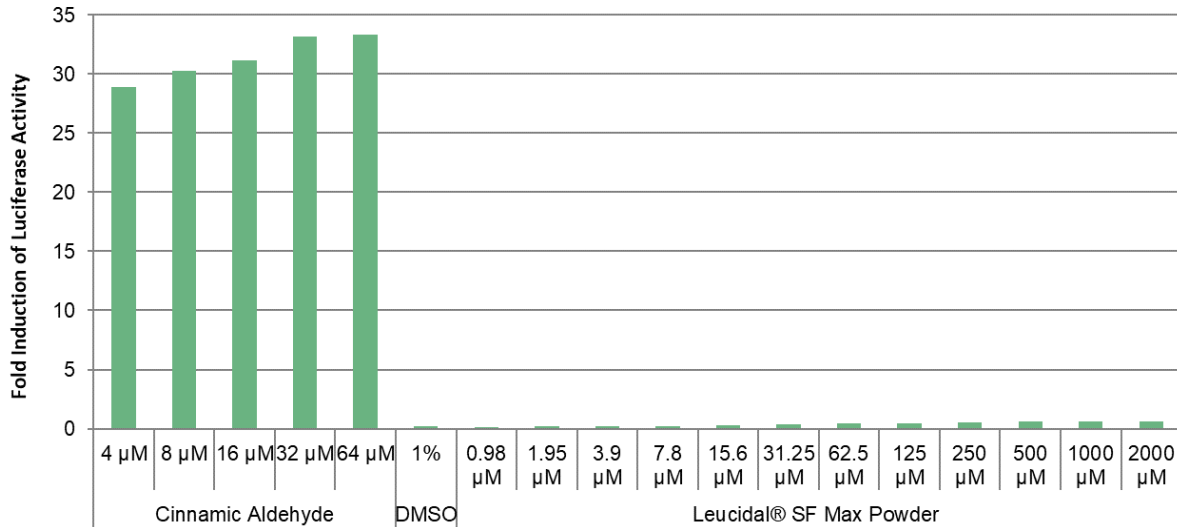


Figure 1: Fold Induction of Luciferase

Discussion

As shown in the results, **Leucidal® SF Max Powder (M15031)** 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® SF Max Powder** can be safely used in cosmetics and personal care products at typical use levels.



Phototoxicity Assay Analysis

107 Technology Drive • Lincolnton, NC 28092
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Code: M15031

CAS #: 1686112-36-6 (or) 68333-16-4

Test Request Form #: 10290

Lot #: 841500

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

Study Director: Maureen Drumwright

Principle Investigator: Daniel Shill

Test Performed:

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

SUMMARY

In vitro phototoxicity irritation studies were conducted to evaluate whether **Leucidal® SF Max Powder** 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 0.5%, 1.5%, 5.0% and 10.0%. 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 four varying concentrations and incubated overnight at 37 °C, 5% CO₂, and 95% relative humidity (RH). The following day, the appropriate tissue inserts were irradiated (UVA) for 60 minutes with 1.7 mW/cm² (=6 J/cm²). 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 0.5%, 1.5%, 5.0%, and 10.0%. 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% CO₂ 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 H₂O
- 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 chlorpromazine, 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/cm² 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% CO₂ 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% CO₂, 95% RH).

C. Tissue Irradiation

Tissue inserts in their 6-well plates are UVA-irradiated for 60 minutes with 6 J/cm² 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% CO₂, 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% CO₂, 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 (OD₅₇₀) 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 0.5%, 1.5%, and 5.0%. 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.

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 0.5%, 1.5%, 5.0% and 10.0%. The negative and positive controls performed as anticipated. There is a slight decrease in viability at the 10% concentration but viability does not decrease more than the acceptable 20%. We can safely say that **Leucidal® SF Max Powder** is not a photoirritant when used at the suggested use levels of 0.50% - 1.00%.

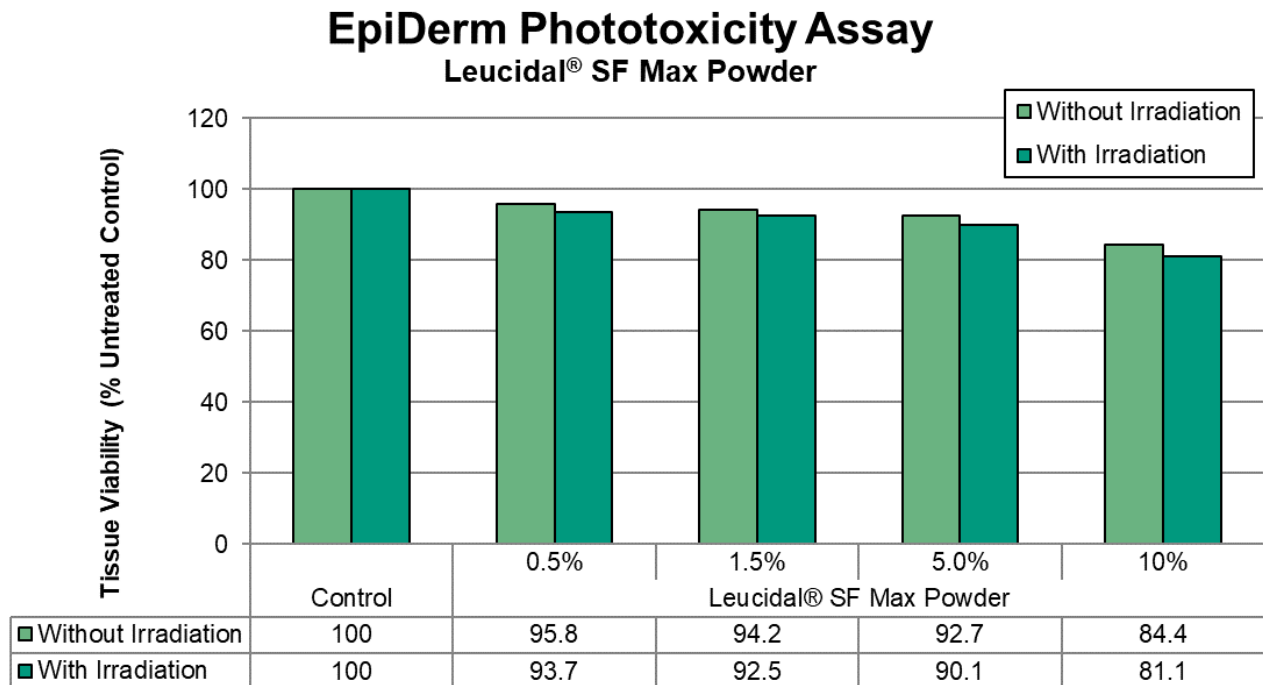


Figure 1: EpiDerm Phototoxicity Graph



Certificate of Origin

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

Leucidal® SF Max Powder Code: M15031

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)

1

Natural Origin Index (NOI)

1



Safety Data Sheet

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Leucidal® SF Max Powder

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Date: 06 / 12 / 2023

Version: 4

Cancels and replaces version: 3

SECTION 1. IDENTIFICATION

Product Name/Identifier	Leucidal® SF Max Powder
Product Code	M15031
Recommended Use	Ingredients/Raw materials used in the manufacturing of Cosmetic Products'; 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

Information contained in this technical literature is believed to be accurate and is offered in good faith for the benefit of the customer. The company, however, cannot assume any liability or risk involved in the use of its chemical products since the conditions of use are beyond our control. Statements concerning the possible use of our products are not intended as recommendations to use our products in the infringement of any patent. We make no warranty of any kind, expressed or implied, other than that the material conforms to the applicable standard specification.



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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

<u>Substance</u>	<u>CAS Numbers</u>	<u>EC Numbers</u>	<u>Percentage</u>
Lactobacillus Ferment	1686112-36-6 (or) 68333-16-4	N/A (or) N/A	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.

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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.

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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: Free flowing powder - Hygroscopic
Color: White to pale yellow

Odor: Characteristic

Loss on Drying (1g/1hr/105°C): 5.0% Maximum

pH (1% Solution in Water): 4.5 – 7.5

Heavy Metals: < 20 ppm
Lead: < 10 ppm
Arsenic: < 2 ppm
Cadmium: < 1 ppm

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Bacteriocins (HPLC): 20.0 – 25.0%

Minimum Inhibitory Concentration

Organism (ATCC#):

E. coli (#8739):	0.12 – 0.25%
S. aureus (#6538):	0.05 – 0.25%
P. aeruginosa (#9027):	0.12 – 0.25%
C. albicans (#10231):	0.12 – 0.25%
A. brasiliensis (#16404):	0.12 – 0.25%

Specific Gravity: Not determined

Vapor density: Not applicable

Boiling Point: Not applicable

Freezing Point: Not applicable

Melting point: Not determined

Flash point: Not applicable

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 Irritation Model)

Ocular: Non-Irritant (Ocular Irritation Model)

Inhalation: Not Determined

Acute toxicity data: Non-Irritant, Non-Primary Sensitizer & Non-Photo irritant

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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: EC₁₀ (Freshwater Alga): 139.24 mg/L - Not harmful to aquatic organisms
EC₂₀ (Freshwater Alga): 258.81 mg/L - Not harmful to aquatic organisms

Biodegradability:

Persistence: Readily Biodegradable (92.4% 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



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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)

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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) N/A
TSCA inventory status:	Exempt	
AICS inventory status:	Not Listed: 1686112-36-6 Listed: 68333-16-4	
Canadian (CEPA DSL) inventory status:	Not Listed: Lactobacillus Ferment (1686112-36-6) Listed as Lactobacillus acidophilus (Revised ICL)	
Japan (MITI list):	Lactobacillus Ferment	
Korea:	Lactobacillus Ferment	
China inventory status:	Lactobacillus Ferment	
Philippines inventory status:	Not Listed: Lactobacillus Ferment (1686112-36-6 (or) 68333-16-4)	

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

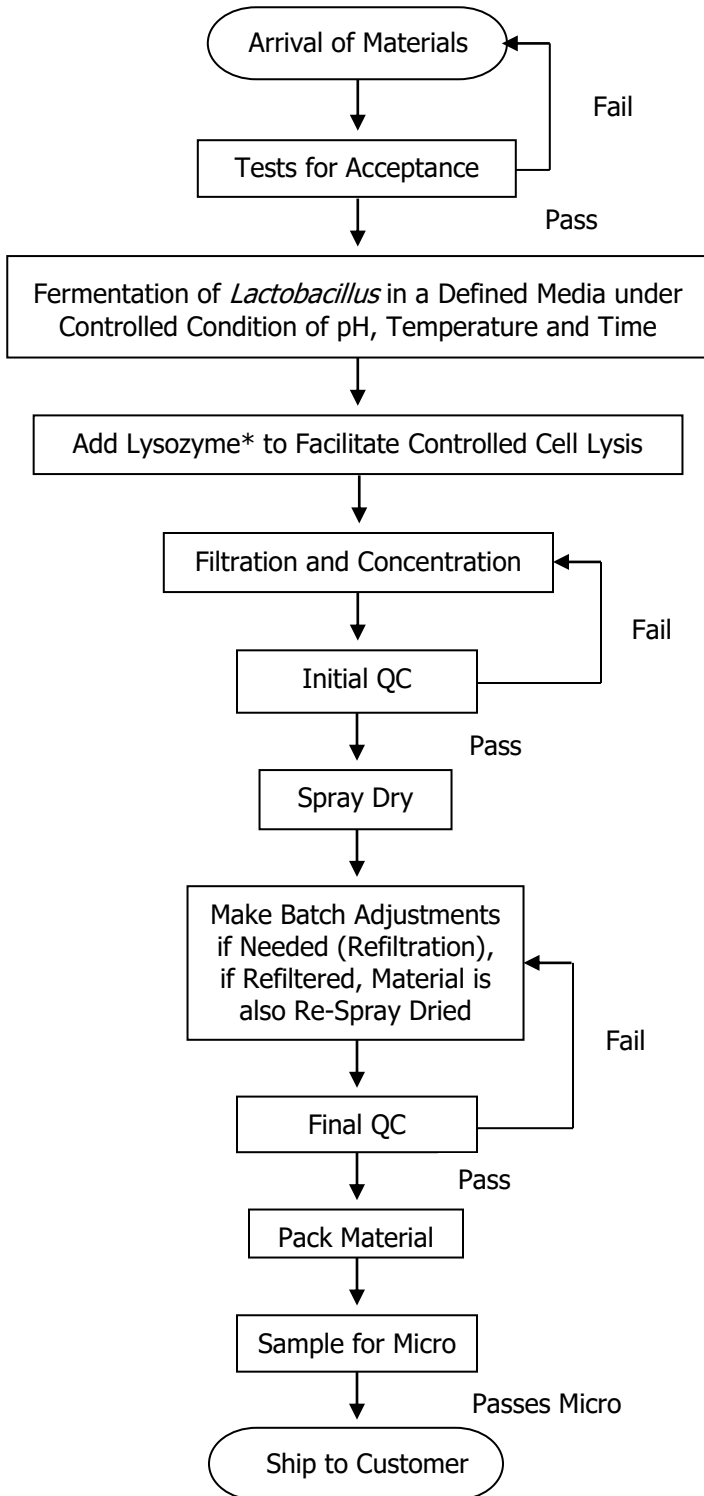
Prohibited uses:	For specific uses, food industry, ask the manufacturer for more information.
Last Revision Date:	05/11/2023
Preparation Date:	06/12/2023
MSDS summary of changes	- Added Biodegradability Data – Section 12 (Ecological Information) - Added Ecotoxicity Data – Section 12 (Ecological Information) - Updated Recommended Use – Section 1 (Identification) & Added Irritation, Acute Toxicity, Sensitization & Mutagenicity Data – Section 11 (Toxicological 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.



M15031-Leucidal® SF Max Powder Manufacturing Flow Chart

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*Lysozyme is derived from Papaya (*Carica papaya*)

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Leucidal® SF Max Powder Certificate of Compliance

Code: M15031
INCI Name: Lactobacillus Ferment
INCI Status: Conforms
CAS #: 1686112-36-6 (or) 68333-16-4
EINECS #: N/A (or) N/A
China NMPA #: 005675-04561-7390

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
USA (TSCA)	Compliant
Australia (AICS)	Compliant
Japan (METI)	Compliant
Canada (DSL)	Compliant
China (IECIC)	Compliant
Brazil (ANVISA)	Compliant
Korea (KECI)	Compliant
Philippines (PICCS)	Contact Us
Mexico (COFEPRIS)	Compliant

Leucidal® SF Max Powder Code: M15031

Attention must be paid to the use of Leucidal® SF Max Powder 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® SF Max Powder 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). The recommended use levels for Leucidal® SF Max Powder is 0.50 – 1.00%.

Leucidal® SF Max Powder 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 audits its suppliers to conform compliance with the Nagoya Protocol where applicable.

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 Concepts, 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® SF Max Powder does not contain any materials prohibited by Halal laws.

As of October 13, 2022, Leucidal® SF Max Powder does not contain any substances present on the so called “candidate list” provided by the European Chemicals Agency (ECHA). We further certify that this material has not been manufactured using any of the species listed in the CITES Appendices as of October 13, 2022.

Leucidal® SF Max Powder is REACH Compliant and free of the following:

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



REACH Compliance Statement

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Trade Name: Leucidal[®] SF Max Powder (M15031)

INCI Name: Lactobacillus Ferment

This is to certify that Leucidal[®] SF Max Powder 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.