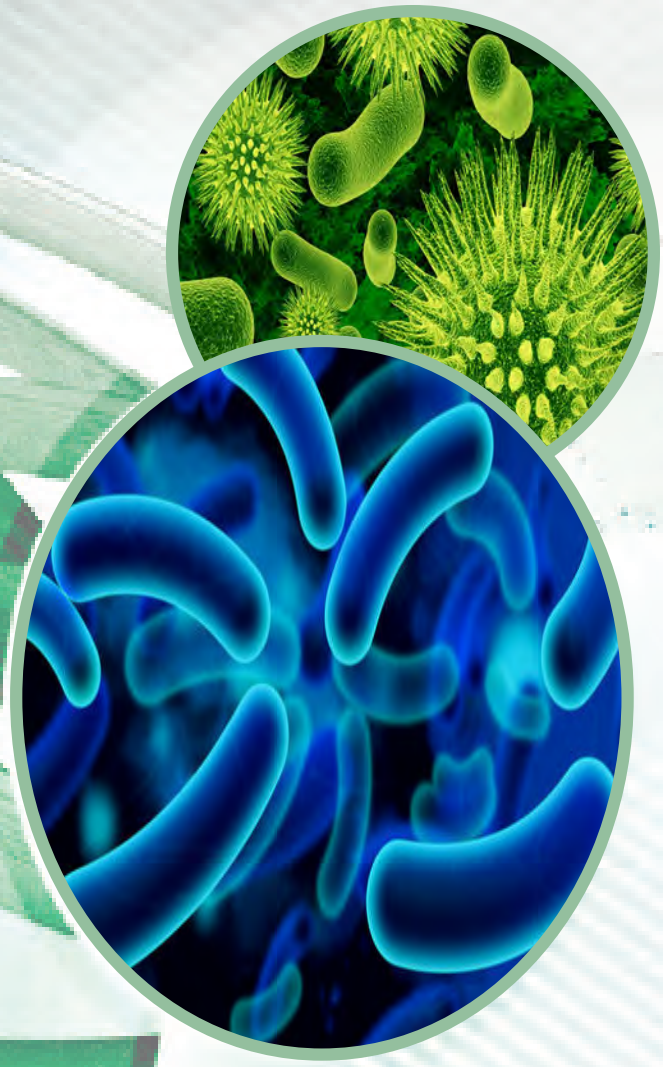
ACTIVE MICRO TECHNOLOGIES

Technical Dossier



ability natural scalp technology Activity
sustainability benefits Redness lactobacillus
moisture Cosmos condition Soothe
Improving solar choice antimicrobial

ProBiocin V™

Code Number: M14005

INCI Name: Lactobacillus Ferment Lysate

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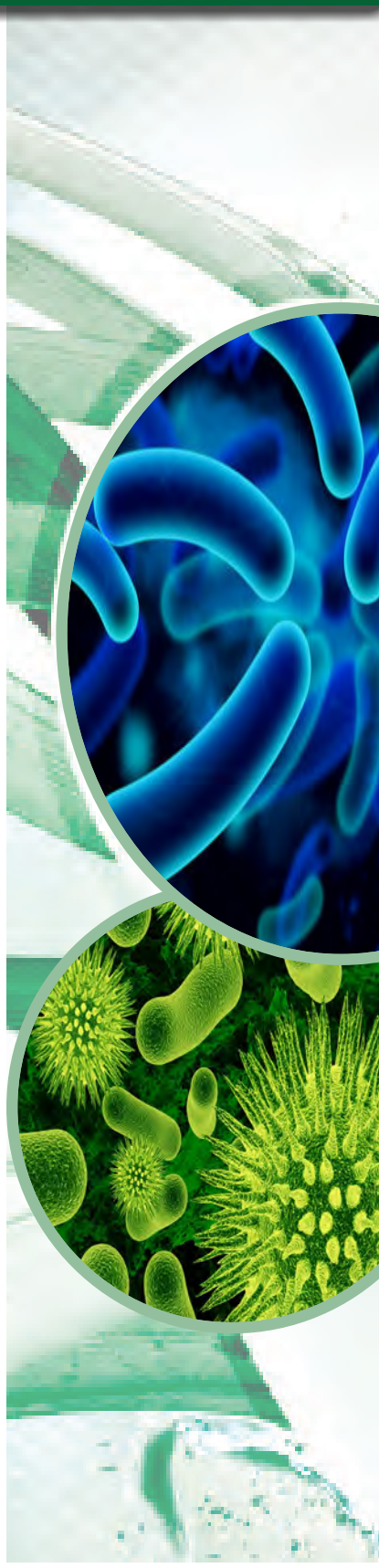

ProBiocin V™ Code Number: M14005
INCI Name: Lactobacillus Ferment Lysate

ProBiocin V™

Technical Data Sheet

BACKGROUND

Scalp care is a prevalent branch of hair care, as a healthy scalp means healthy hair growth. However, dandruff has been shown to effect approximately 50% of the population, regardless of gender or ethnicity[1], driving consumer's interests in products that can restore balance to an "unhealthy" scalp. *Malassezia spp.* has been shown to play a key role in the pathogenesis of dandruff. A study performed on a group of dandruff patients resulted in 84% of participants having a species of *Malassezia* present on the scalp[2]. Inspired by dandruff related irritation, research has driven product development to address *Malassezia* and balance the scalp microbiome.

The culturing of the probiotic bacterium *Lactobacillus acidophilus*, supported by prebiotic nutrients, diminishes the presence of *Malassezia* on the scalp, while presenting a new global approach to non-traditional preservative technologies. **ProBiocin V™** harnesses the benefits of prebiotic oligosaccharides to offer a competitive edge to commensal microorganisms in their natural ecosystem. This optimized production method of supplementing the growth media aids in the production of postbiotic bacteriocin peptides, capable of delivering moisturizing and redness reduction benefits to soothe an irritated scalp.

Current efforts to reduce the prevalence of microorganisms associated with dandruff, involve the development of scalp and hair care products able to alleviate caustic pathogenic microorganisms, while relieving the scalp from itchiness. Traditional preservatives, frequently used in hair care applications, have been shown to be detrimental to both commensal and pathogenic bacteria living on the skin and scalp.

Code Number: M14005
INCI Nomenclature:
Lactobacillus Ferment Lysate
INCI Status: Conforms
REACH Status: Compliant
CAS Number: 68333-16-4 [AICS, Revised ICL] (or) 92128-79-5 [PICCS]
EINECS Number: N/A (or) 295-777-8
Origin: Biotechnology
Lactobacillus
Processing:
GMO Free
No Ethoxylation
No Irradiation
No Sulphonation
No Ethylene Oxide treatment
No Hydrogenation
Additives: None
Preservatives: None
Antioxidants: None
Other additives: None
Solvents used: Water
Appearance: Clear to Slightly Hazy, Colorless to Yellow Liquid
Soluble/Miscible: Water Soluble
Suggested Use Levels: 2.0 - 4.0%
Suggested Applications:
Antimicrobial, Redness Reduction, Scalp Care

Consumer concerns regarding the use of synthetic preservative systems, such as parabens, formaldehyde donors and phenoxyethanol[3], have pushed formulators to seek out alternative means of preservation that can provide broad spectrum antimicrobial protection and a multifunctional activity. Consumers often perceive traditional preservative systems as being associated with sensitization[4]. **ProBiocin V™** is a technology able to diminish these concerns, while providing efficacious benefits against scalp irritation. The

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ProBiocin V™

produced bacteriocin peptides are also capable of providing broad spectrum antimicrobial protection in order to maintain the integrity of the formulation. **ProBiocin V™** acts as a multifunctional system that boosts preservation while delivering soothing benefits to the scalp. **ProBiocin V™** is also vegan and compliant in many global markets.

Veganism is a popular trend in the cosmetic industry as well. As consumers are making lifestyle choices that make them feel healthier, the same logic applies to the products they use. Although you do not have to be vegan to use vegan products, consumers are beginning to pay more attention to what goes into the products they use and how these products will benefit them long-term. While shifting towards animal alternatives and away from ethical concerns, sacrificing the quality of ingredients is not an option when it comes to treating common cosmetic concerns such as moisturization and redness.

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

SCIENCE

Lactobacillus acidophilus, harnessing probiotic attributes, produces postbiotic antimicrobial peptides known as bacteriocins[5]. To achieve this, prebiotic chicory oligosaccharide fructans are supplemented into the *Lactobacillus* growth media during fermentation. Lysozyme is then used to facilitate controlled cell lysis of the ferment filtrate. When applied to the scalp, beneficial microorganisms are enriched and the microbiota is modulated, in effort to achieve a homeostatic environment. The *Lactobacillus* derived peptides work to condition the scalp, while reducing pathogenic microorganisms to soothe irritation.

BENEFITS

Acting as a multifunctional system that boosts preservation while delivering soothing benefits to the scalp, **ProBiocin V™** utilizes the properties of prebiotic oligosaccharides as an approach to postbiotic bacteriocin procurement to deliver scalp moisturization and redness reduction.

The ability of **ProBiocin V™** to inhibit the growth of a variety of bacteria and fungi was determined using the Minimum Inhibitory Concentration (MIC) test. The results are illustrated in Figure 1, showing that this material provides broad spectrum antimicrobial protection.

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

Figure 1. MIC data for **ProBiocin V™**.

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The positive MIC screening results warranted further testing to confirm its ability to provide product preservation. Double Challenge Tests were completed using either 2.0% or 4.0% **ProBiocin V™** in a generic cream base formulation at pH values of 3, 5, and 7. Samples were inoculated with *E. coli, P. aeruginosa*, *S. aureus*, *C. albicans*, and *A. brasiliensis*. A challenge test against *Malasezzia furfur* was also completed using 4% **ProBiocin V™** in a generic shampoo formulation.

During the first 28-day incubation period, samples were periodically collected and tested for the presence of these microorganisms. Following this initial 28 days of incubation, the cream and shampoo samples were then re-inoculated with the microbial cultures and sampled over an additional 28-day period. Figure 2 shows the positive preservation results for **ProBiocin V™** tested at 4.0% in a generic cream base formulation at pH 5. Figure 3 shows the reduction of viable organism Malassezia furfur using **ProBiocin V™** tested at 4.0% in a generic shampoo formulation.

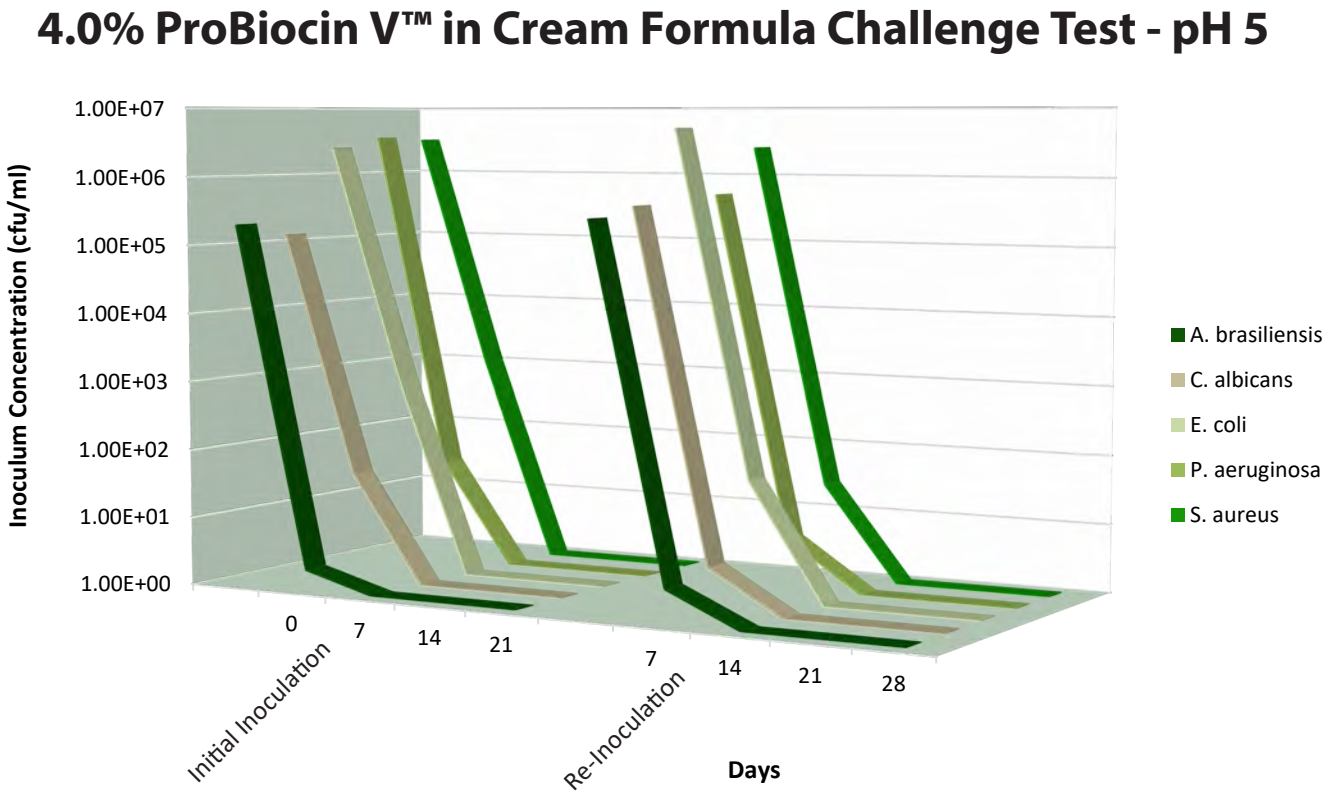

Figure 2. Challenge Test results for Generic Cream Formula pH 5 with 4.0% **ProBiocin V™** inoculated on Day 0 and re-inoculated on Day 28. Results show log reduction in viable organisms.

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Organism	Sampling Interval				
Inoculum Initial (CFU/ml)	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Malassezia furfur 5.6 x 10^5	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Re-innoculation (CFU/ml)	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Malassezia furfur 2.5 x 10^5	N/A	>99.999%	>99.999%	>99.999%	>99.999%

Figure 3. Challenge Test results for Generic Shampoo Formula with 4% **ProBiocin V™** on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

A Time Kill Test was performed to determine the change in population of aerobic microorganisms within a specified sampling time when tested against 4.0% **ProBiocin V™** solution. The activity of the test material inoculated was evaluated at determine time intervals of 30 seconds, 1, 5, 10 and 30 minutes after the inoculation to determine quantitatively the number of viable microorganisms remaining after the incubation time. As shown in Figure 4, the Gram-positive and Gram-negative bacteria as well as the yeast and mold were reduced by 99.9% within 30 seconds interval of the test after the inoculation.

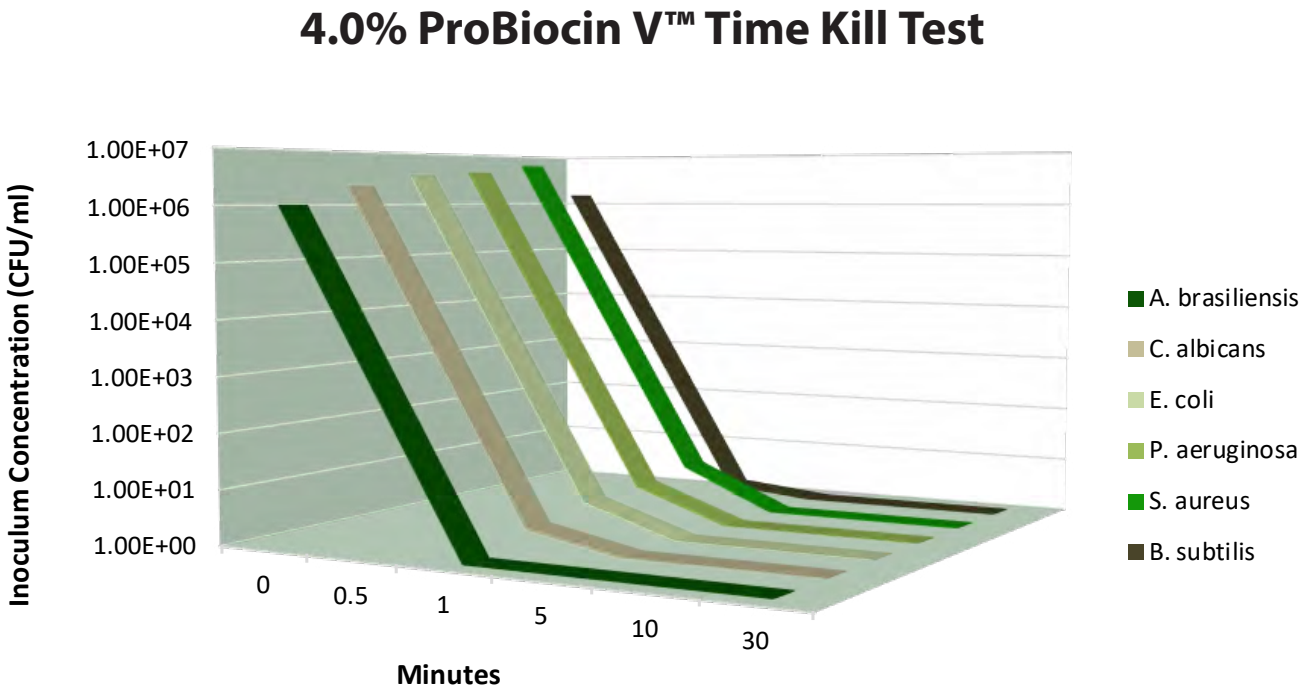

Figure 4. Time Kill Test results for 4.0% **ProBiocin V™**.

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ProBiocin V™

A salon study was conducted to determine the scalp care benefits of 4.0% **ProBiocin V™** in a shampoo and conditioner vs. the base shampoo and conditioner alone. The study was conducted using 10 panelists and each panelist had a baseline photo taken of his or her hairline prior to beginning the study. Panelist's heads were treated with either the base shampoo and conditioner or the experimental containing 4.0% **ProBiocin V™** in the base shampoo and base conditioner. After the application and rinse of the experimental and control applications, each panelist's hair was blown dry using a round brush. Each panelist had his or her head washed three times per week for a two week period. After the third wash of the week, panelists had scalp moisturization and pigmentation values recorded.

Throughout the treatment period, participants using 4.0% **ProBiocin V™** in a base shampoo and conditioner displayed an overall increase in moisturization and reduction in erythema when compared to the control group using the base shampoo and conditioner alone. The experimental (4.0% **ProBiocin V™** in shampoo and conditioner) demonstrated directional results as P-values were shown to be less than 5%, and statistically proved to be significant when comparing the overall moisturization and erythema averages of the experimental and control groups.

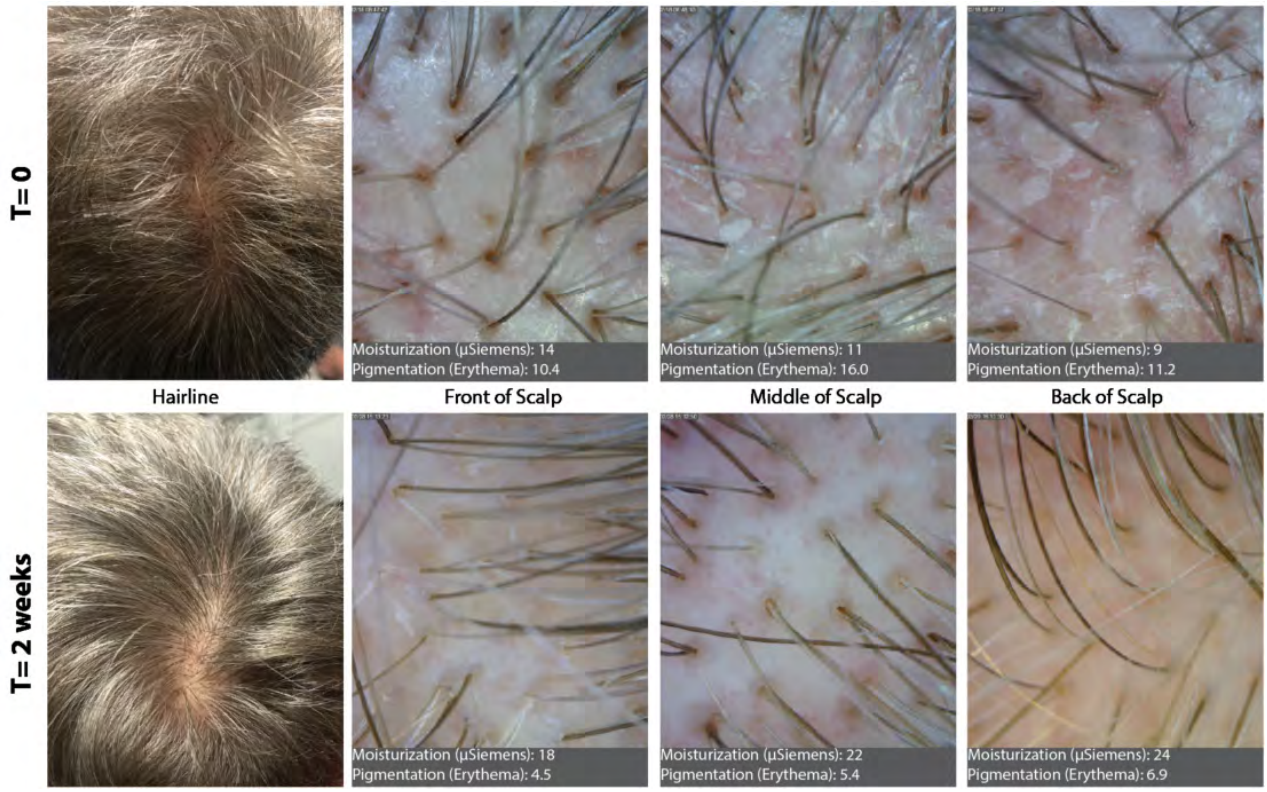

Figure 5. Images of Panelist #4 treated with 4.0% ProBiocin V™ in Base Shampoo and Conditioner display an increase in overall scalp moisturization (front, middle, back averages) by 88.26% and decrease in overall scalp erythema (front, middle, back averages) by 55.31% from beginning of treatment (T=0) to T=2 Weeks via DermaLab analysis.

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The ability of **ProBiocin V™** to inhibit the growth of *Malasezzia furfur*, in comparison to industry leading anti-fungals, was determined using the Minimum Inhibitory Concentration (MIC) test. The results are illustrated in Figure 6, showing that **ProBiocin V™** provides comparable anti-fungal protection to Piroctone Olamine, while out performing Climbazole.

Product Name	MIC (%) to Inhibit *M. furfur*
ProBiocin V™	1.00
Climbazole	2.00
Piroctone Olamine	1.00
Zinc Pyrithione	0.01

Figure 6. *M. furfur* MIC comparison data.

An *in-vivo* VISIA® analysis was conducted over a period of six weeks to evaluate the effects of 4.0% **ProBiocin V™** in a base lotion on red area parameters compared to the base lotion alone. **ProBiocin™ V** demonstrated the ability to provide a reduction in red area feature counts by 24.35% after four weeks of treatment when compared to the control. Two weeks after treatment ceased, **ProBiocin™ V** continued to provide red area reduction by 13.81% when compared to the control.

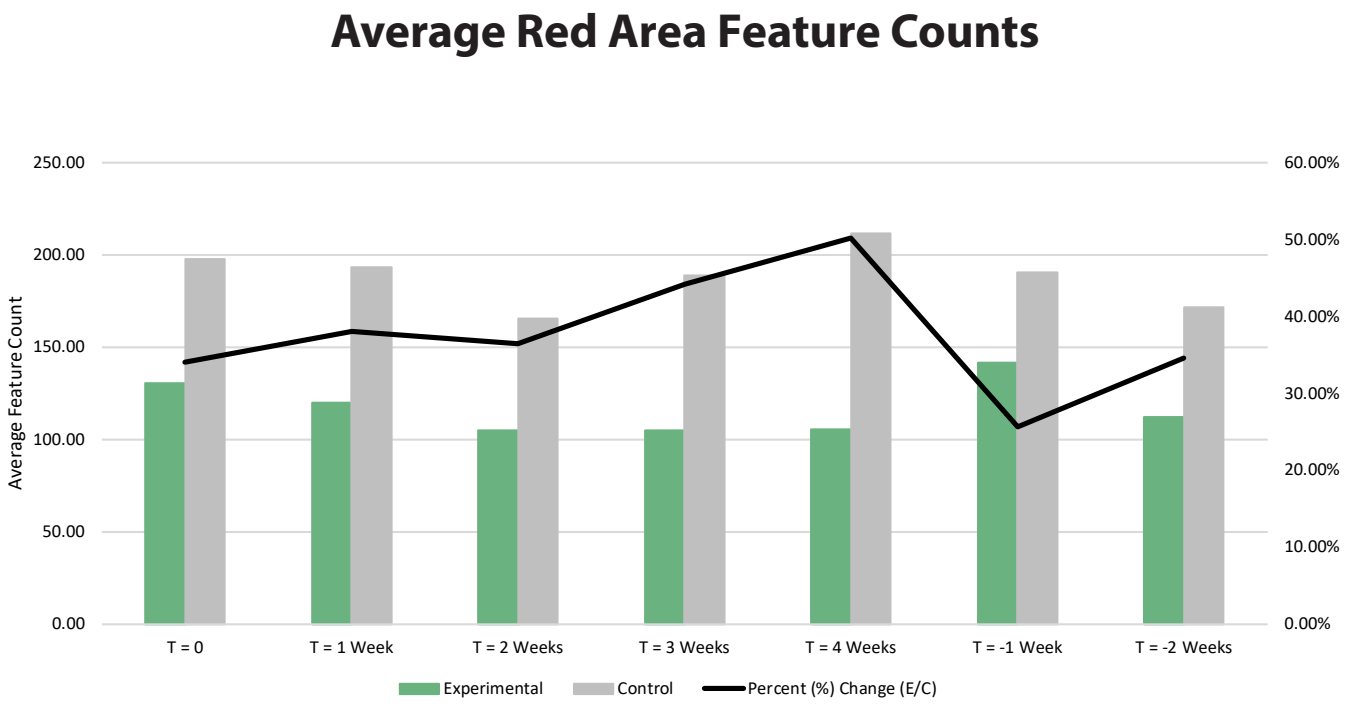

Figure 7. Reduction in front face red areas at various time points with a trendline of percent change, in feature counts, between the experimental and control values at time points.

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Figure 8. Panelist #3 treated with 4.0% ProBiocin™ V in Base Lotion displays a reduction (25.8%) in feature counts for red areas from beginning of treatment (T=0) to T=4 Weeks via VISIA Image Analysis. Images on the left are panelist #3 with image enhancement, through VISIA, which provides higher visualization of feature changes. Images on the right are natural photos of panelist #3.

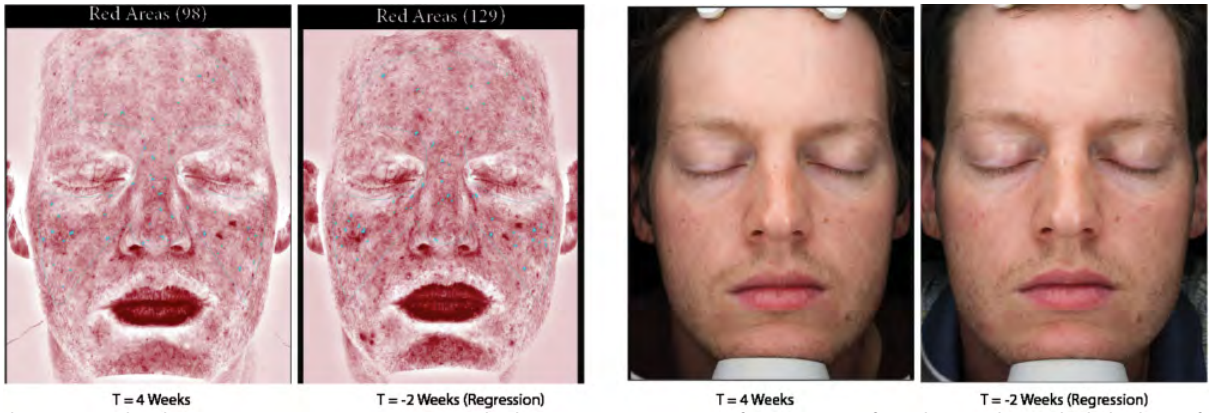

Figure 9. Panelist #3 treated with 4.0% ProBiocin™ V in Base Lotion displays a increase (31.6%) in feature counts for red areas (due to the halted use of product) from 4 weeks to -2 weeks (regression) via VISIA Image Analysis. Images on the left are panelist #3 with image enhancement, through VISIA, which provides higher visualization of feature changes. Images on the right are natural photos of panelist #3.

USE RECOMMENDATIONS

ProBiocin V™ 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 8, during the cooling phase of the process at temperatures lower than 40°C.

References

1. Ranganathan, S., and T. Mukhopadhyay. "Dandruff: The most commercially exploited skin disease." Indian journal of dermatology 55.2 (2010): 130.
2. Rudramurthy, Shivaprakash M., et al. "Association of Malassezia species with dandruff." The Indian journal of medical research 139.3 (2014): 431.
3. Lee, Eun-Young, et al. "A Study of Influencing Factors for Sensory Irritation Due to Preservatives of Cosmetics." Journal of the Society of Cosmetic Scientists of Korea, Society of Cosmetic Scientists of Korea, www.koreascience.or.kr/article/JAKO200616419600251.page.
4. Kabara, Jon J., and Donald S. Orth. Preservative-Free and Self-Preserving Cosmetics and Drugs: Principles and Practices. Marcel Dekker, 1997.
5. Goh, Yong Jun, and Todd R Klaenhammer. "Genetic Mechanisms of Prebiotic Oligosaccharide Metabolism in Probiotic Microbes." Annual Review of Food Science and Technology, U.S. National Library of Medicine, 2015, www.ncbi.nlm.nih.gov/pubmed/25532597.

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Specification

Product Name: ProBiocin V™
Code Number: M14005
CAS #'s: 68333-16-4 [AICS, Revised ICL] (or) 92128-79-5 [PICCS]
EINECS #'s: N/A (or) 295-777-8
INCI Name: Lactobacillus Ferment Lysate

Specification	Parameter
Appearance	Clear to Slightly Hazy Liquid
Color	Colorless to Yellow
Odor	Characteristic
Solids (1g/1hr/105°C)	20.0 – 25.0%
pH (Direct)	4.0 – 11.0
Heavy Metals	< 20 ppm
Lead	< 10 ppm
Arsenic	< 2 ppm
Cadmium	< 1 ppm
Bacteriocins (HPLC)	5.00 – 10.00%
Minimum Inhibitory Concentration[1]	
Organism (ATCC#)	
E. coli (#8739)	0.25 – 1.00%
S. aureus (#6538)	0.25 – 1.00%
P. aeruginosa (#9027)	0.25 – 1.00%
C. albicans (#10231)	0.25 – 1.00%
A. brasiliensis (#16404)	0.25 – 1.00%

DO NOT FREEZE; Store at or near room temperature;
May sediment upon standing; Mix well prior to use

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

Note:

1) Refer to Inhibition Activity Data

ACTIVE MICRO TECHNOLOGIES

Compositional Breakdown

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ProBiocin V™
Code: M14005

Compositional Breakdown:

Ingredient	%
Lactobacillus Ferment Lysate	100.00

Compositional Breakdown

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

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

Compositional Breakdown

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

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

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This is to certify that ProBiocin V™ does not contain, neither directly nor through cross contamination, any of the 26 allergenic flavors or fragrances (Gas Chromatography-Mass Spectrometer Coupled):

ALLERGENS listed in Annex III of EU Cosmetic Regulation(EC) No. 1223/2009 amending EU Directive 2003/15/EC		
INCI NAME	CAS NUMBER	Limit (ppm)
Alpha-IsoMethyl Ionone	127-51-5	< 0.02
Amyl Cinnamal	122-40-7	< 0.10
Anise Alcohol	105-13-5	< 0.00
Benzyl Alcohol	100-51-6	< 0.01
Benzyl Benzoate	120-51-4	< 0.09
Benzyl Cinnamate	103-41-3	< 0.30
Benzyl Salicylate	118-58-1	< 0.06
Butylphenyl Methylpropional	80-54-6	< 0.50
Cinnamal	104-55-2	< 0.01
Cinnamyl Alcohol	104-54-1	< 0.30
Citral	5392-40-5	< 1.00
Citronellol	106-22-9	< 1.00
Coumarin	91-64-5	< 0.00
Eugenol	97-53-0	< 0.70
Farnesol	4602-84-0	< 0.04
Geraniol	106-24-1	< 0.08
Hexyl Cinnamal	101-86-0	< 0.40
Hydroxycitronellal	107-75-5	< 1.00
Hydroxymethylpentyl 3-Cyclohexene carboxaldehyde	31906-04-4	< 0.00
Isoeugenol	97-54-1	< 0.06
Limonene	5989-27-5	< 0.05
Linalool	78-70-6	< 0.00
Methyl 2-Octynoate	111-12-6	< 0.20
Evernia prunastri extract (Oak moss extract)	90028-68-5	< 0.00
Evernia furfuracea extract (Treemoss extract)	90028-67-4	< 0.00
Amylcinnamyl Alcohol	101-85-9	< 1.00

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This is to certify that ProBiocin V™ does not contain pesticide levels exceeding the following (Reverse Phase High Performance Liquid Chromatography-Mass Spectrometer Coupled):

EPA Pesticide Levels	
INCI NAME	LIMIT (mg/kg)
Alachlor	< 0.02
Aldrin and Dieldrin	< 0 .05
Azinphos-methyl	< 1. 00
Bromopropylate	< 3.0 0
Chlordane(cis and trans)	< 0.05
Chlorfenvinphos	< 0.50
Chlorpyrifos	< 0.20
Chlorpyrifos-methyl	< 0.10
Cypermethrin	< 1.00
DDT	< 1.00
Deltamethrin	< 0.50
Diazinon	< 0.50
Dichlorvos	< 1.00
Dithiocarbamates	< 2.00
Endosulfan	< 3.00
Endrin	< 0.05
Ethion	< 2.00
Fenitrothion	< 0.50
Fenvalerate	< 1.50
Fonofos	< 0.05
Heptachlor	< 0.05
Hexachlorobenzene	< 0.10
Hexachlorocyclohexane	< 0.30
Lindane	< 0.60
Malathion	< 1.00
Methidathion	< 0.20

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Parathion	< 0.50
Parathion-methyl	< 0.20
Permethrin	< 1.00
Phosalone	< 0.10
Piperonyl butoxide	< 3.00
Pirimiphos-methyl	< 4.00
Pyrethrins	< 3.00
Quintozene(sum of 3 items)	< 1.00

ProBiocin V™

INTRODUCTION

An *in-vivo* study was conducted over a period of six weeks to evaluate the effects of 4.0% **ProBiocin V™** in a base lotion on red area parameters compared to the base lotion alone. **ProBiocin V™** demonstrated the ability to provide a reduction in red area feature counts by 24.35% after four weeks of treatment when compared to the control. Two weeks after treatment ceased, **ProBiocin V™** continued to provide red area reduction by 13.81% when compared to the control.

MATERIALS AND METHODS

This study was conducted using 10 M/F participants between the ages of 23 - 55. Each participant was instructed to apply 2.0 mg of lotion to their entire face twice a day for a four week period. Participants were instructed to continue their usual skin care routine and to apply the lotion once their everyday skin care routine is finished. Half of the participant population used 4.0% **ProBiocin V™** in a Cetaphil Daily Facial Moisturizer for all skin types, while the other half used the Cetaphil Daily Facial Moisturizer alone as a control.

Baseline photos were taken prior to starting the lotion regimen. Photos were taken once a week for 6 weeks, with four weeks being the regular testing period and the final two weeks being the regression period where application has ceased. Female participants were instructed to not wear makeup during the testing period. Photographic assessments were performed using the VISIA Complexion Analysis System (Canfield Scientific., Fairfield, NJ, USA). The VISIA System, with a configurable head support, ensured consistent positioning of each subject's head. The subjects cleaned their skin with a gentle facial wipe (Daily Facial Towelettes – Paraben Free Formula by Kirkland Signature) before the image was obtained. The photographic images were captured with standard, cross-polarized, parallel polarized, and ultraviolet light. Images were taken for each subject to quantify the feature counts for red areas.

Feature counts provide a count of the number of discrete instances of the feature being evaluated. Skin with a lower feature count was considered to be more youthful in appearance. In the present study, scores were used to more objectively assess changes in skin condition. The average scores for the front face were calculated, and the differences between time points were recorded and compared. For statistical analysis a two-sample t-test, assuming unequal variance, was performed to compare data. The significance threshold was set at 0.05.

Code Number: M14005

INCI Name: Lactobacillus Ferment Lysate
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CAS Number: 68333-16-4 [AICS, Revised ICL] (or) 92128-79-5 [PICCS]
EINECS Number: N/A (or) 295-777-8

TRF#: 6035
Lot Number(s): N191209F

Suggested Use Levels: 2.0 - 4.0%
Use Level for Assay: 4.0%

Sponsor:
Active Concepts, LLC
107 Technology Drive
Lincolnton, North Carolina 28092

Study Director: Maureen Danaher
Principle Investigator: Kara Rivera

Suggested Applications:
Conditioning, Antimicrobial

Benefits of **ProBiocin V™**:

- Salicylate Free
- Antimicrobial
- Moisturization

ProBiocin V™

RESULTS

Reduction in red areas were determined throughout the four week treatment period and the two week regression period, after treatment ceased. Figure 1 illustrates the reduction in front face red areas throughout the study and depicts the percent change, in feature counts, between the experimental and control at various time points. Statistical analysis performed compares the feature count averages of the experimental and control feature counts throughout the duration of the study. Figure 2 displays the p-values for red area feature counts highlighted in yellow. Figure 3 details the average feature counts for red areas during the study for experimental and control participants as well as the percent change at each time point. The VISIA Complexion Analysis System provides photographic assessments with image enhancements to provide higher visualization of feature changes. Figures 4-9 provide visualizations of red area feature changes throughout the study period on participants using **ProBiocin V™**. Selected time periods, in Figures 4-9, are shown with both natural photographs and VISIA enhanced images of each participant. Red area feature counts were collected for experimental and control groups during the study. The averages were calculated as well as the percent change between each time point for both experimental and control values. The result of the t-Test, in terms of p-value, was 6.3517E -07 and 1.27034E -06, respectively.

Average Red Area Feature Counts

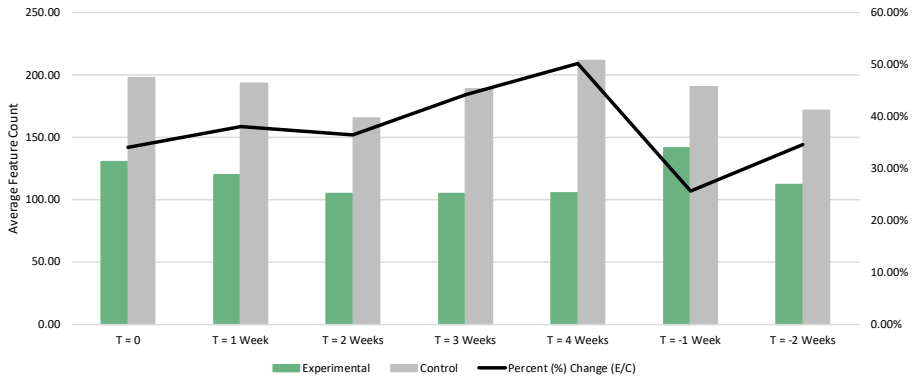


Figure1. Reduction in front face red areas at various time points with a trendline of percent change, in feature counts, between the experimental and control values at time points.

Red Areas Statistical Analysis

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	117.2	188.6285714
Variance	206.96	245.8457143
Observations	7	7
Hypothesized Mean Difference	0	
df	12	
t Stat	-8.88106472	
P(T<=t) one-tail	6.3517E-07	
t Critical one-tail	1.782287556	
P(T<=t) two-tail	1.27034E-06	
t Critical two-tail	2.17881283	

Figure 2. Statistical analysis on the overall face feature count averages comparing the percent change of experimental and control values throughout treatment to their respective baselines.

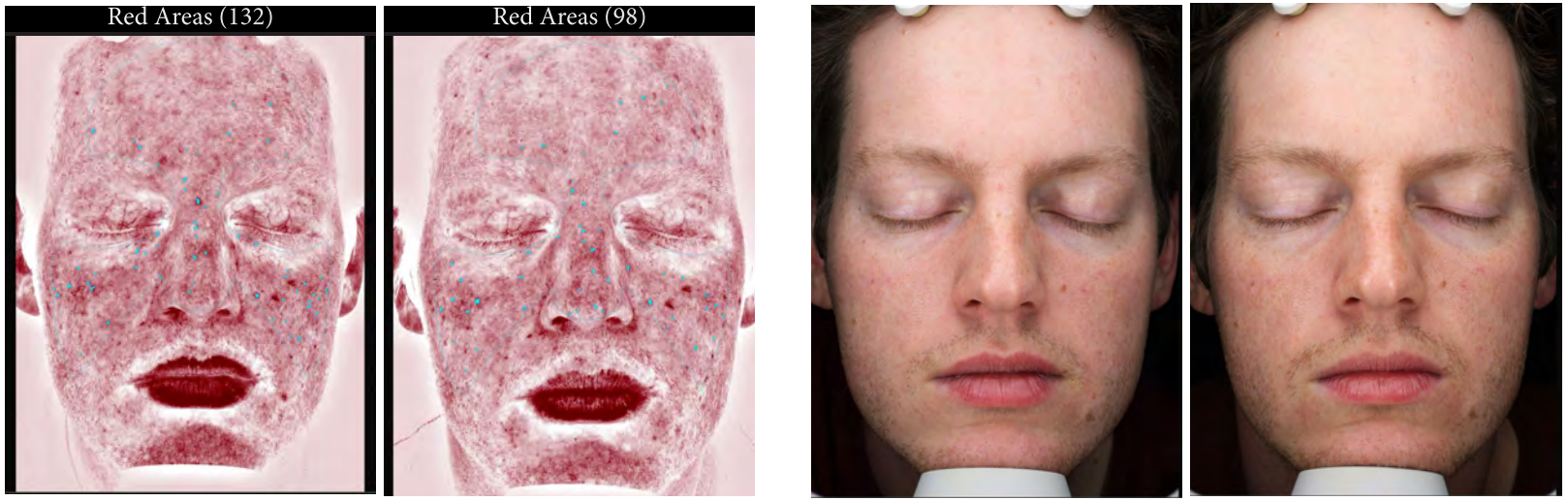
Average Feature Counts for Red Areas

	Front Face Feature Count Averages						
Experimental	143.00	140.73	134.07	125.13	135.80	141.13	133.60
Control	167.73	162.33	144.93	157.73	179.50	161.20	155.00
	T = 0	**T = 1 Week**	**T = 2 Weeks**	**T = 3 Weeks**	**T = 4 Weeks**	**T = -1 Week**	**T = -2 Weeks**
Percent (%) Change E/C	14.75%	13.31%	7.50%	20.67%	24.35%	12.45%	13.81%

Figure 3. Feature counts for red areas determined using front face averages of experimental and control groups. Percent change is determined by comparing the experimental front face averages with control front face averages at each time point.

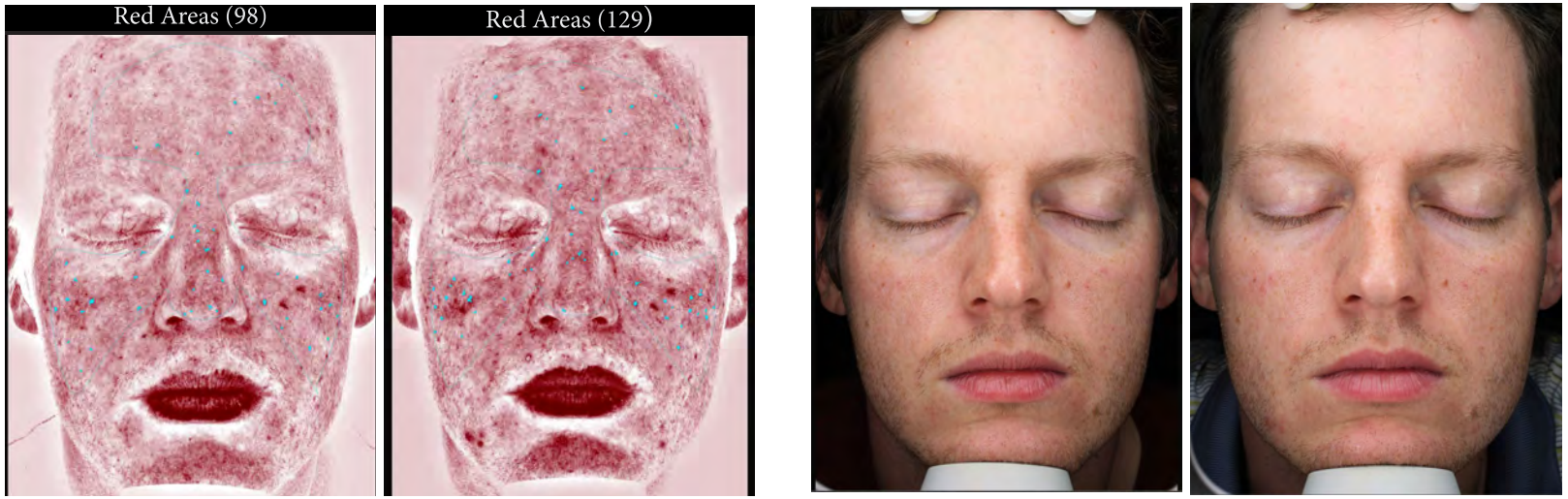
ProBiocin V™

RESULTS



T = 0 T = 4 Weeks T = 0 T = 4 Weeks

Figure 4. Panelist #3 treated with 4.0% **ProBiocin V™** in Base Lotion displays a reduction (25.8%) in feature counts for red areas from beginning of treatment (T=0) to T=4 Weeks via VISIA Image Analysis. Images on the left are panelist #3 with image enhancement, through VISIA, which provides higher visualization of feature changes. Images on the right are natural photos of panelist #3.



T = 4 Weeks T = -2 Weeks (Regression) T = 4 Weeks T = -2 Weeks (Regression)

Figure 5. Panelist #3 treated with 4.0% **ProBiocin V™** in Base Lotion displays a increase (31.6%) in feature counts for red areas (due to the halted use of product) from 4 weeks to -2 weeks (regression) via VISIA Image Analysis. Images on the left are panelist #3 with image enhancement, through VISIA, which provides higher visualization of feature changes. Images on the right are natural photos of panelist #3.

ProBiocin V™

RESULTS

T = 0 T = 4 Weeks T = 0 T = 4 Weeks

Figure 6. Panelist #4 treated with 4.0% **ProBiocin V™** in Base Lotion displays a reduction (35.0%) in feature counts for red areas from beginning of treatment (T=0) to T=4 Weeks via VISIA Image Analysis. Images on the left are panelist #4 with image enhancement, through VISIA, which provides higher visualization of feature changes. Images on the right are natural photos of panelist #4.

T = 4 Weeks T = -2 Weeks (Regression) T = 4 Weeks T = -2 Weeks (Regression)

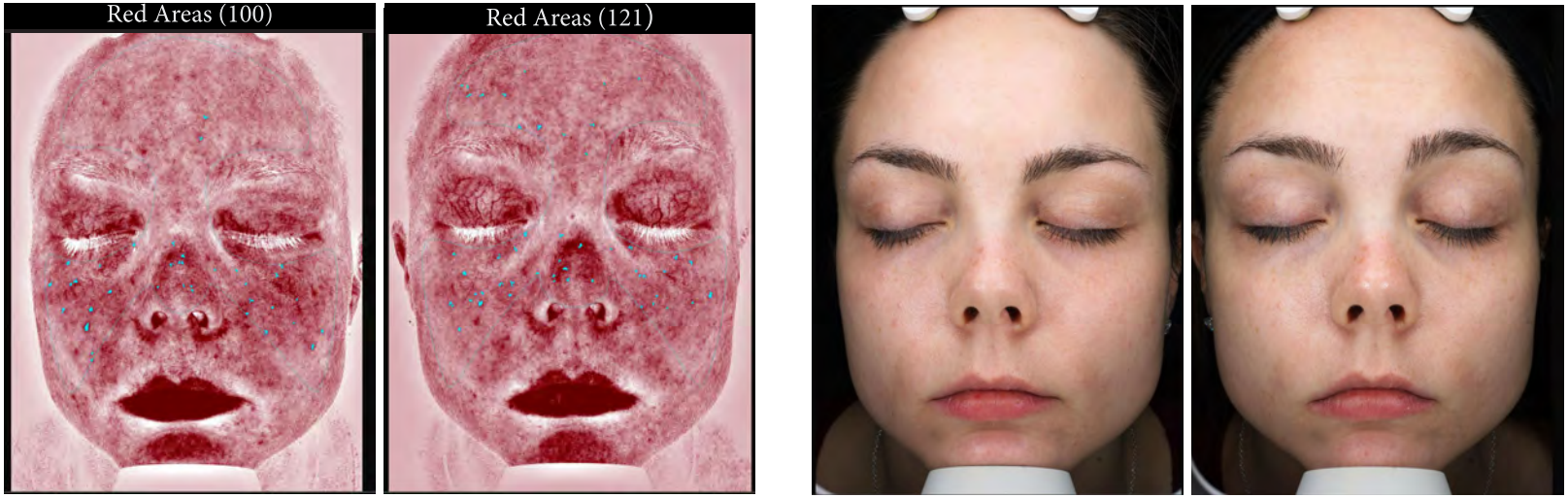
Figure 7. Panelist #4 treated with 4.0% **ProBiocin V™** in Base Lotion displays an increase (24.5%) in feature counts for red areas (due to the halted use of product) from 4 weeks to -2 weeks (regression) via VISIA Image Analysis. Images on the left are panelist #4 with image enhancement, through VISIA, which provides higher visualization of feature changes. Images on the right are natural photos of panelist #4.

ProBiocin V™

RESULTS

T = 0 T = 4 Weeks T = 0 T = 4 Weeks

Figure 8. Panelist #5 treated with 4.0% **ProBiocin V™** in Base Lotion displays a reduction (42.2%) in feature counts for red areas from beginning of treatment (T=0) to T=4 Weeks via VISIA Image Analysis. Images on the left are panelist #5 with image enhancement, through VISIA, which provides higher visualization of feature changes. Images on the right are natural photos of panelist #5.



T = 4 Weeks T = -2 Weeks (Regression) T = 4 Weeks T = -2 Weeks (Regression)

Figure 9. Panelist #5 treated with 4.0% **ProBiocin V™** in Base Lotion displays an increase (21.0%) in feature counts for red areas (due to the halted use of product) from 4 weeks to -2 weeks (regression) via VISIA Image Analysis. Images on the left are panelist #5 with image enhancement, through VISIA, which provides higher visualization of feature changes. Images on the right are natural photos of panelist #5.

ProBiocin V™

DISCUSSION

Digital photographs and facial surface analysis were conducted as objective computer assessments by VISIA Complexion Analysis. Improvements in red areas were evaluated by comparing feature counts throughout the course of treatment. 4.0% **ProBiocin V™** in base lotion demonstrated the ability to reduce the average feature count of red areas by 24.35% after four weeks of treatment application. Following two weeks of regression, 4.0% **ProBiocin V™** in base lotion demonstrated the ability to reduce the average feature count of red areas by 13.81%. The active in base lotion demonstrated directional results as P-values were shown to be less than 5%, and statistically proved to be significant when comparing the feature count average of experimental and control groups.

Throughout the treatment period, participants using **ProBiocin V™** in base lotion displayed an overall reduced number of red areas when compared to the control group. Some participants experienced an increase in red area feature counts during the regression period, which would be expected as treatment has ceased. Other participants experienced a reduction in red area feature counts during the regression period, indicating a lasting effect after treatment.

Skin protecting and correcting trends are now taking center stage. Redness is common in problematic skin, but we are able to fight the red areas by incorporating products into our skin care routines that reduce visible signs of redness. **ProBiocin V™** is a probiotic inspired ingredient capable of reducing skin redness.

Active Concepts, LLC
Lincolnton, NC. USA
www. activeconceptsllc.com
Office: +1 (704) 276 7100
info@activeconceptsllc.com

Active Concepts S.r.l.
Milano ITALY
www.activeconcepts.it
Tel +39 02 90360719
info@activeconcepts.it

Active Concepts LLC, Asia
Kaohsiung, Taiwan
www.activeconceptsllc.com
Tel + 886 73599900
info@activeconceptsllc.com.tw

Active Concepts Japan
Tokyo, Japan
www.activeconceptsllc.com
Tel + 813 62311022
info-tokyo@activeconceptsllc.com

ProBiocin V™

INTRODUCTION

A series of *in-vivo* studies were conducted over the course of 2 weeks to evaluate the scalp care benefits of **ProBiocin V™**. In order to determine if **ProBiocin V™** is suitable for scalp care, panelists participated in a salon study combined with a moisturization assesment and a pigmentation assessment. Ten panelists were recruited to use test shampoos and conditioners three times per week for 2 weeks after a one week washout phase. The moisturization assay was conducted to assess the hydrating ability of **ProBiocin V™** in a shampoo and conditioner formulation. The pigmentation assay was conducted to assess the ability of **ProBiocin V™** to reduce redness caused by erythema. Throughout the treatment period, participants using 4.0% **ProBiocin V™** in a base shampoo and conditioner displayed an overall increase in moisturization and reduction in erythema when compared to the control group using the base shampoo and conditioner alone. The experimental (4.0% **ProBiocin V™** in shampoo and conditioner) demonstrated directional results as P-values were shown to be less than 5%, and statistically proved to be significant when comparing the overall moisturization and erythema averages of the experimental and control groups.

MATERIALS AND METHODS

A salon study was conducted to determine the scalp care benefits of 4.0% **ProBiocin V™** in a shampoo and conditioner vs. the base shampoo and conditioner alone. The study was conducted using 10 panelists and each panelist had a baseline photo taken of his or her hairline prior to beginning the study. Panelist's heads were treated with either the base shampoo and conditioner or the experimental containing 4.0% **ProBiocin V™** in the base shampoo and base conditioner. After the application and rinse of the experimental and control applications, each panelist's hair was blown dry using a round brush. Each panelist had his or her head washed three times per week for a two week period. After the third wash of the week, panelists had scalp moisturization and pigmentation values recorded.

The DermaLab 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 Dermalab Corneometer was used to measure the moisture levels on each panelist's scalp. 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 day one of the study. Panelists had moisturization readings taken of the scalp after the third wash of each week for a two week period.

The pigmentation measurement of the DermaLab Combo is performed using a handheld probe. This probe accommodates the color sensor, filters, optics, and light source. The light source is composed of two high intensity white LEDs, as well as a guiding light, which illuminates the target during positioning of the probe. Once the probe is in place, the LEDs flash at full power to illuminate the target area. Erythema levels were then measured and recorded. Baseline pigmentation readings were taken on day one of the study. Panelists had pigmentation readings taken of the scalp after the third wash of the week for two weeks.

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Code Number: M14005

INCI Name: Lactobacillus Ferment Lysate
INCI Status: Conforms
REACH Status: Complies
CAS Number: 68333-16-4 [AICS, Revised ICL] (or) 92128-79-5 [PICCS]
EINECS Number: N/A (or) 295-777-8

TRF#: 6052
Lot Number(s): S200210E

Suggested Use Levels: 2.0 - 4.0%
Use Level for Assay: 4.0%

Sponsor:
Active Concepts, LLC
107 Technology Drive
Lincolnton, North Carolina 28092

Study Director: Maureen Danaher
Principle Investigator: Parisa Mehrzadeh

Suggested Applications:
Moisturizing, Antimicrobial

Benefits of **ProBiocin V™:**

- Moisturization
- Antimicrobial
- Erythema Reduction
- Significant p-values

ProBiocin V™

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RESULTS

Figure 1 illustrates the average moisturization levels throughout the study and depicts the percent change between the experimental and control at each time points. As evidenced in this 2 week efficacy study of **ProBiocin V™** on the scalp, moisture levels were improved by 42.29% after one week and by 58.54% after 2 weeks when compared to the control. Results indicate that 4.0% **ProBiocin V™** is capable of increasing scalp moisturization when compared to the control shampoo and conditioner alone. Statistical analysis performed compares the overall moisturization averages of the experimental and control throughout the duration of the study. Figure 2 displays the p-values for scalp moisturization highlighted in yellow. For statistical analysis a two-sample t-test, assuming unequal variance, was performed to compare data. The significance threshold was set at 0.05. Figure 3 details the average overall scalp moisturization values during the study and percent change at each time point.

Comparative Scalp Moisturization

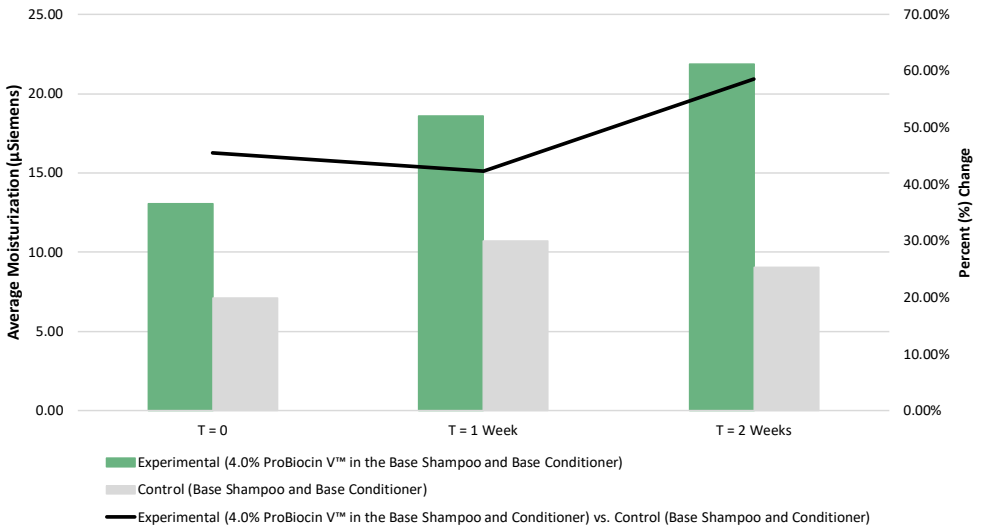

Figure 1. Overall (front, middle, back) scalp moisturization averages at each time point with a trendline of percent change between the experimental and control values.

Statistical Analysis Scalp Moisturization

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	17.8444444	8.97037037
Variance	19.7881481	3.28707819
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	3.19971054	
P(T<=t) one-tail	0.02467138	
t Critical one-tail	2.35336343	
P(T<=t) two-tail	0.04934277	
t Critical two-tail	3.18244631	

Figure 2. Statistical analysis on the overall (front, middle, back) scalp moisturization averages comparing the percent change of experimental and control values throughout treatment.

Overall Scalp Moisturization

	Overall Scalp Moisturization Averages		
	T = 0	**T = 1 Week**	**T = 2 Weeks**
Experimental (4.0% ProBiocin V™ in the Base Shampoo and Base Conditioner)	13.07	18.60	21.87
Control (Base Shampoo and Base Conditioner)	7.11	10.73	9.07
Percent Change	**T = 0**	**T = 1 Week**	**T = 2 Weeks**
Experimental (4.0% ProBiocin V™ in the Base Shampoo and Conditioner) vs. Control (Base Shampoo and Conditioner)	45.58%	42.29%	58.54%

Figure 3. Overall (front, middle, back) scalp moisturization averages and percent change between the experimental and control values at each time point.

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RESULTS

Figure 4 illustrates the average erythema levels throughout the study and depicts the percent change between the experimental and control at each time points. As evidenced in this 2 week efficacy study of **ProBiocin V™** on the scalp, erythema levels were reduced by 122.68% after one week and by 211.70% after 2 weeks when compared to the control. Results indicate that 4.0% **ProBiocin V™** is capable of reducing scalp erythema when compared to the control shampoo and conditioner alone. Statistical analysis performed compares the overall erythema averages of the experimental and control throughout the duration of the study. Figure 5 displays the p-values for scalp erythema highlighted in yellow. For statistical analysis a two-sample t-test, assuming unequal variance, was performed to compare data. The significance threshold was set at 0.05. Figure 6 details the average overall scalp erythema values during the study and percent change at each time point.

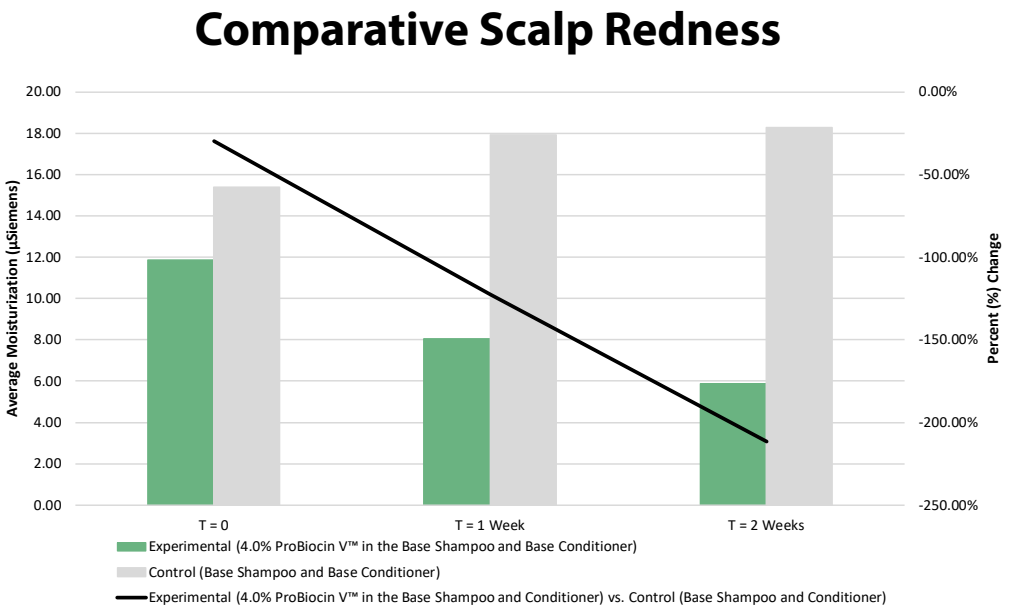

Figure 4. Overall (front, middle, back) scalp erythema averages at each time point with a trendline of percent change between the experimental and control values.

Statistical Analysis Scalp Redness

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	8.59644444	17.2066667
Variance	9.22922904	2.47924444
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-4.3583776	
P(T<=t) one-tail	0.01116157	
t Critical one-tail	2.35336343	
P(T<=t) two-tail	0.02232313	
t Critical two-tail	3.18244631	

Figure 5. Statistical analysis on the overall (front, middle, back) scalp erythema averages comparing the percent change of experimental and control values throughout treatment.

Overall Scalp Redness

	Overall Scalp Erythema Averages		
	T = 0	**T = 1 Week**	**T = 2 Weeks**
Experimental (4.0% ProBiocin V™ in the Base Shampoo and Base Conditioner)	11.87	8.05	5.87
Control (Base Shampoo and Base Conditioner)	15.40	17.93	18.29
Percent Change	**T = 0**	**T = 1 Week**	**T = 2 Weeks**
Experimental (4.0% ProBiocin V™ in the Base Shampoo and Conditioner) vs. Control (Base Shampoo and Conditioner)	-29.75%	-122.68%	-211.70%

Figure 6. Overall (front, middle, back) scalp erythema averages and percent change between the experimental and control values at each time point.

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RESULTS - IMAGES

Panelist #1 - Experimental Group at T=0 and T=2 weeks

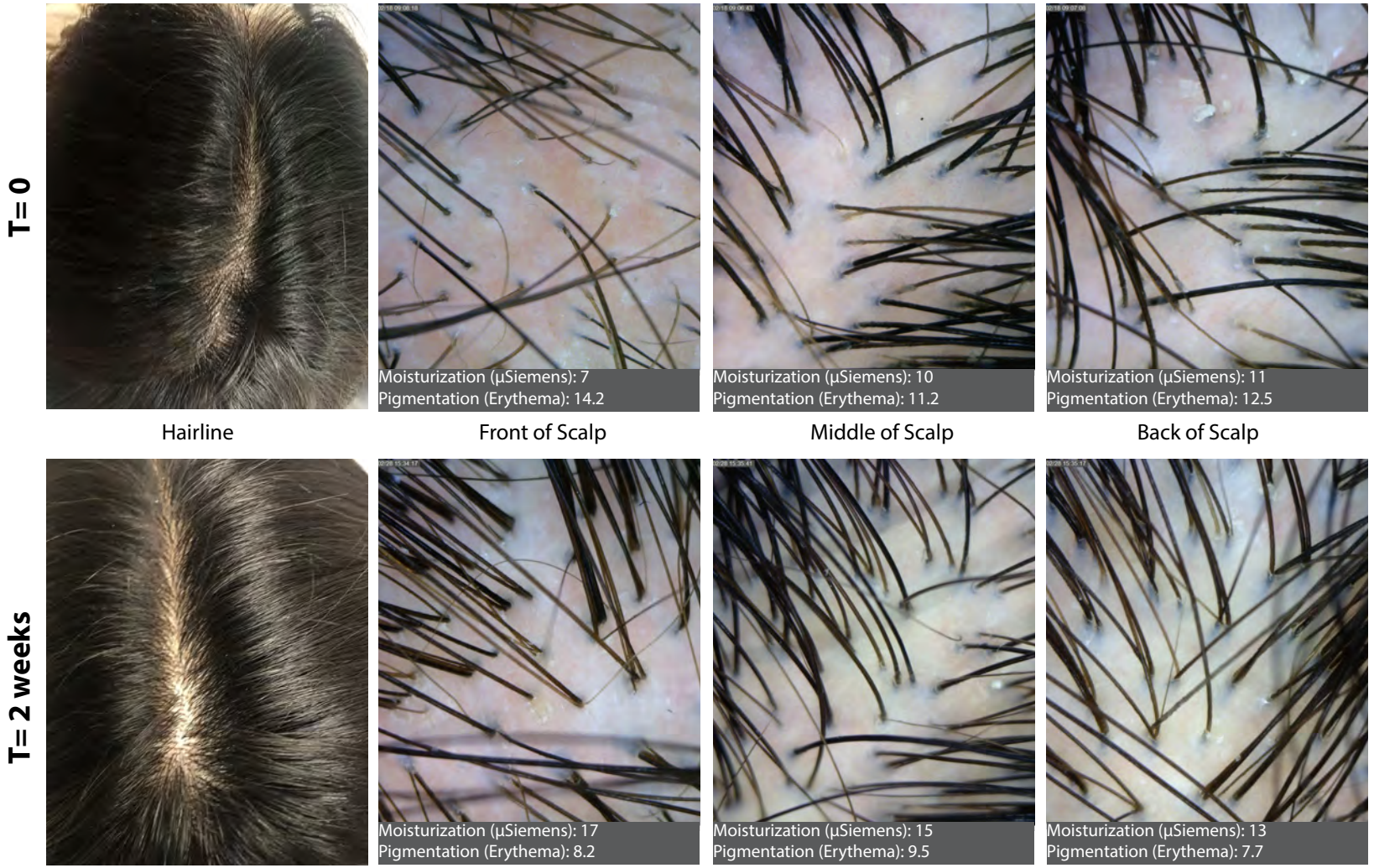

Figure 7. Images of Panelist #1 treated with **4.0% ProBiocin V™** in Base Shampoo and Conditioner display an increase in overall scalp moisturization (front, middle, back averages) by 60.77% and decrease in overall scalp erythema (front, middle, back averages) by 32.94% from beginning of treatment (T=0) to T=2 Weeks via DermaLab analysis.

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RESULTS - IMAGES

Panelist #2 - Experimental Group at T=0 and T=2 weeks

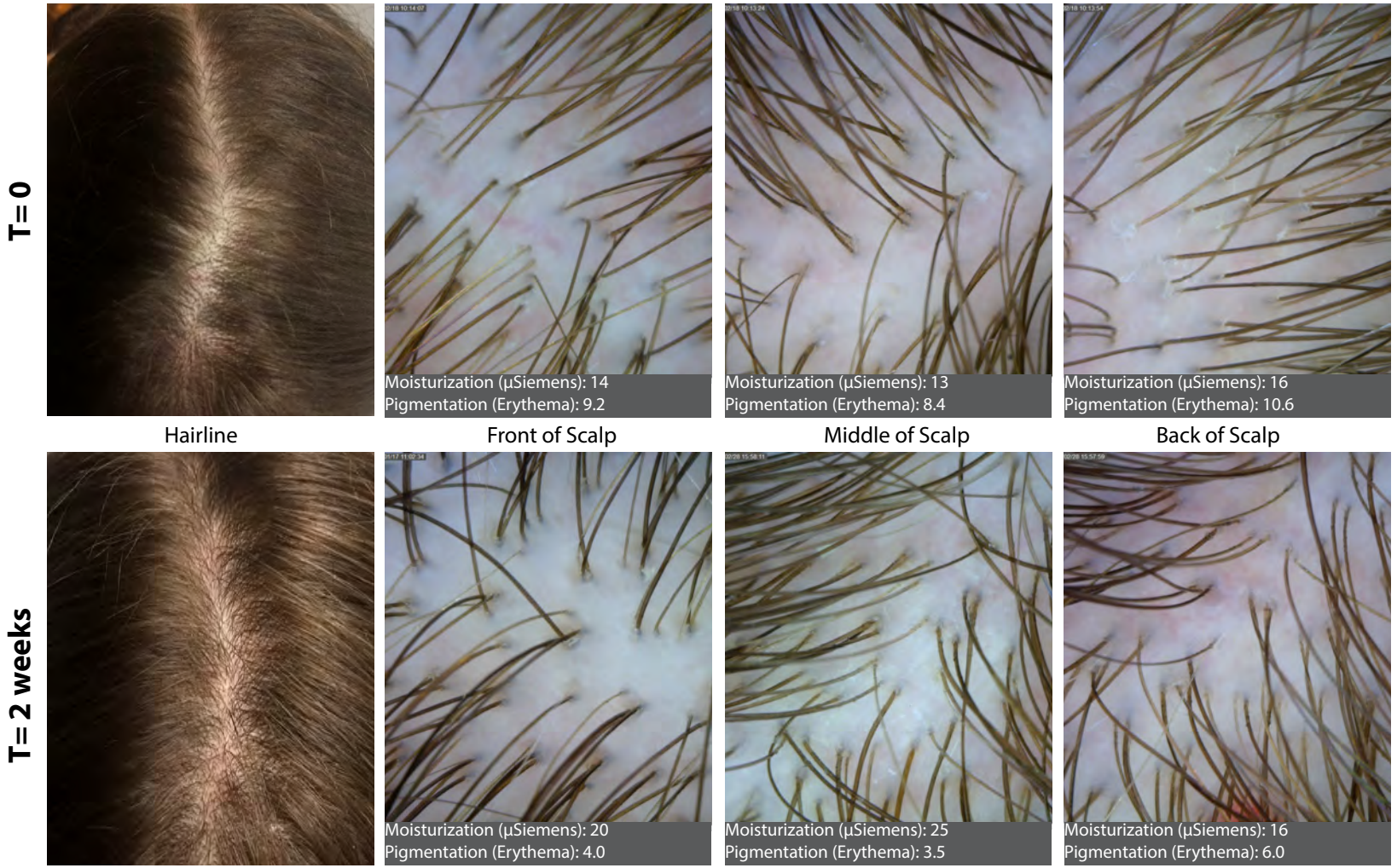

Figure 8. Images of Panelist #2 treated with **4.0% ProBiocin V™** in Base Shampoo and Conditioner display an increase in overall scalp moisturization (front, middle, back averages) by 41.87% and decrease in overall scalp erythema (front, middle, back averages) by 52.13% from beginning of treatment (T=0) to T=2 Weeks via DermaLab analysis.

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RESULTS - IMAGES

Panelist #3 - Experimental Group at T=0 and T=2 weeks

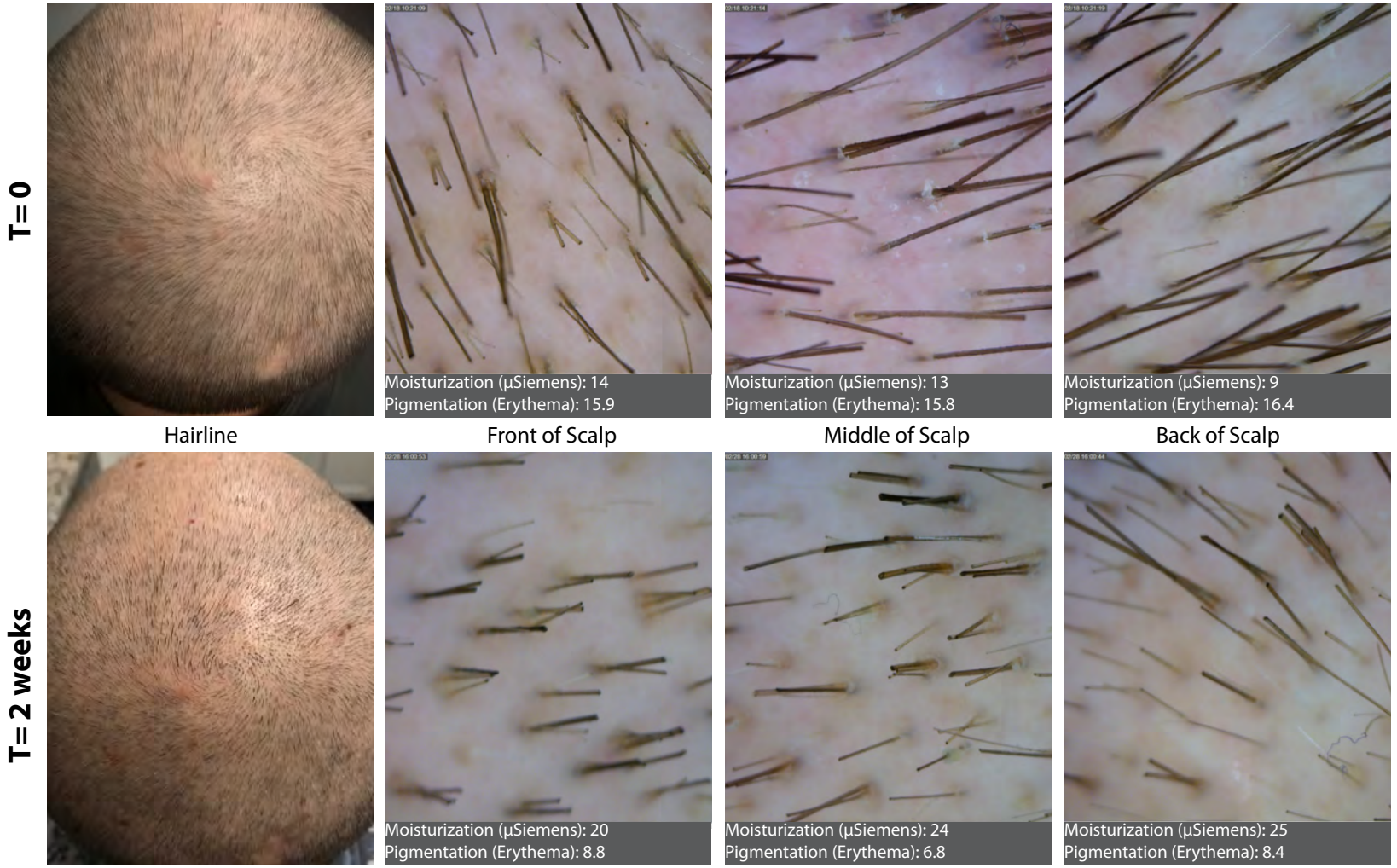

Figure 9. Images of Panelist #3 treated with **4.0% ProBiocin V™** in Base Shampoo and Conditioner display an increase in overall scalp moisturization (front, middle, back averages) by 91.67% and decrease in overall scalp erythema (front, middle, back averages) by 50.09% from beginning of treatment (T=0) to T=2 Weeks via DermaLab analysis.

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RESULTS - IMAGES

Panelist #4 - Experimental Group at T=0 and T=2 weeks

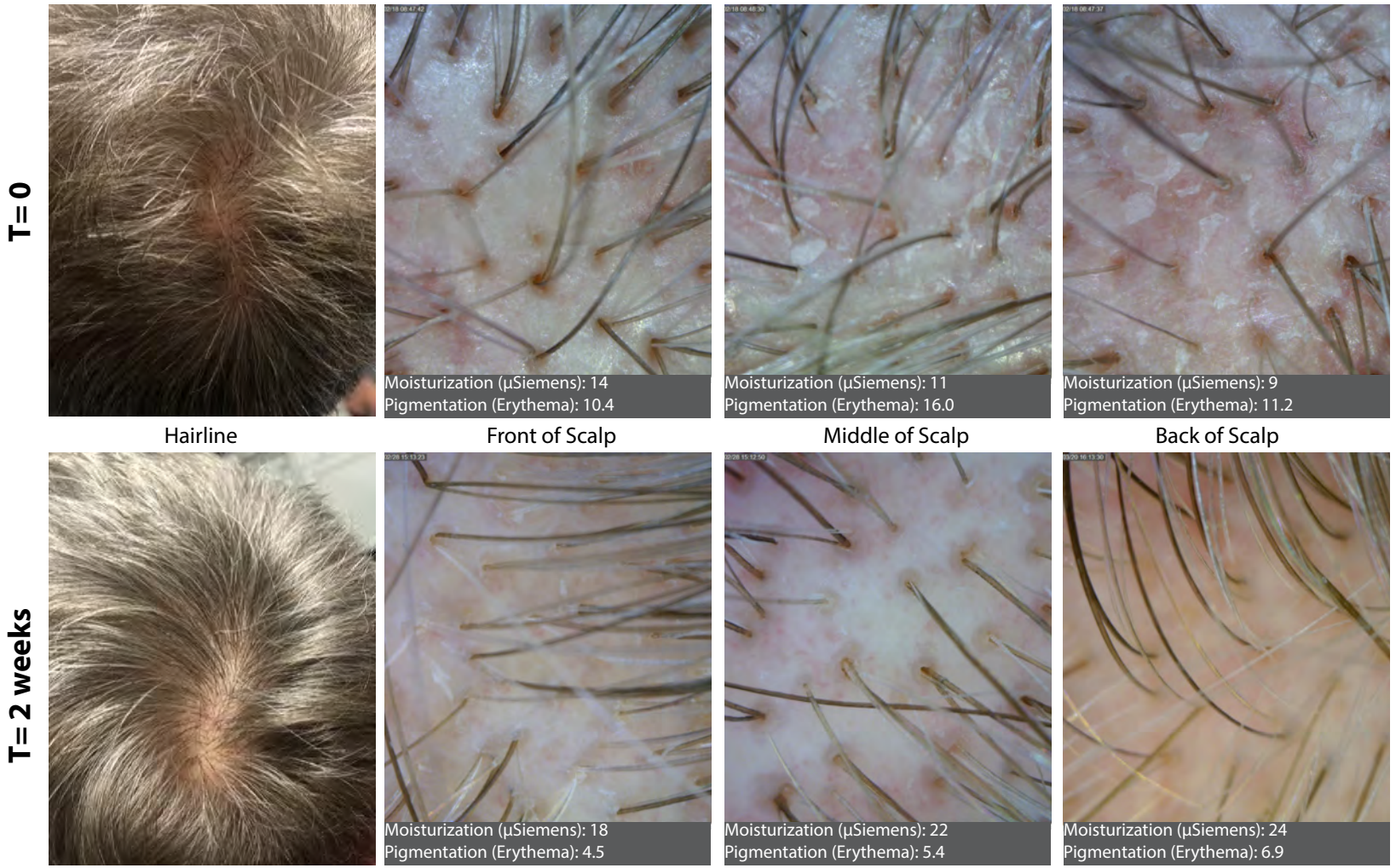

Figure 10. Images of Panelist #4 treated with **4.0% ProBiocin V™** in Base Shampoo and Conditioner display an increase in overall scalp moisturization (front, middle, back averages) by 88.26% and decrease in overall scalp erythema (front, middle, back averages) by 55.31% from beginning of treatment (T=0) to T=2 Weeks via DermaLab analysis.

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RESULTS - IMAGES

Panelist #5 - Experimental Group at T=0 and T=2 weeks

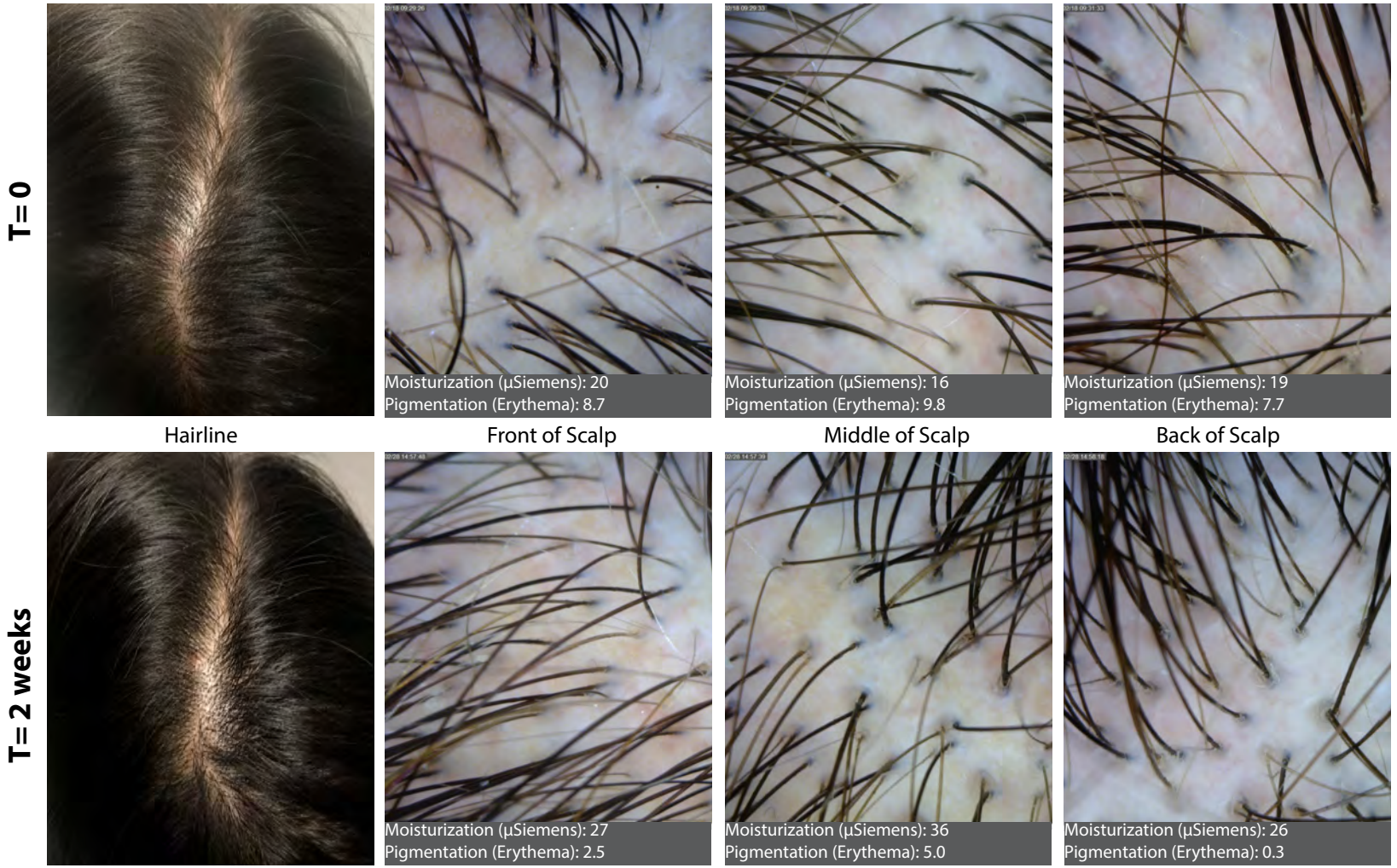

Figure 11. Images of Panelist #5 treated with **4.0% ProBiocin V™** in Base Shampoo and Conditioner display an increase in overall scalp moisturization (front, middle, back averages) by 61.87% and decrease in overall scalp erythema (front, middle, back averages) by 70.22% from beginning of treatment (T=0) to T=2 Weeks via DermaLab analysis.

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DISCUSSION

Throughout the treatment period, participants using 4.0% **ProBiocin V™** in a base shampoo and conditioner displayed an overall increase in moisturization and reduction in erythema when compared to the control group using the base shampoo and conditioner alone. The experimental (4.0% **ProBiocin V™** in shampoo and conditioner) demonstrated directional results as P-values were shown to be less than 5%, and statistically proved to be significant when comparing the overall moisturization and erythema averages of the experimental and control groups.

ProBiocin V™ capitalizes on the rising trend of veganism in the cosmetic and personal care industry and provides exceptional moisturization, redness reduction, and antimicrobial benefits in a wide array of formulations. The present study supports the use of **ProBiocin V™** in scalp care applications.

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Active Concepts, LLC
Lincolnton, NC. USA
www. activeconceptsllc.com
Office: +1 (704) 276 7100
info@activeconceptsllc.com

Active Concepts S.r.l.
Milano ITALY
www.activeconcepts.it
Tel +39 02 90360719
info@activeconcepts.it

Active Concepts LLC, Asia
Kaohsiung, Taiwan
www.activeconceptsllc.com
Tel + 886 73599900
info@activeconceptsllc.com.tw

Active Concepts Japan
Tokyo, Japan
www.activeconceptsllc.com
Tel + 813 62311022
info-tokyo@activeconceptsllc.com

ACTIVE MICRO TECHNOLOGIES

Inhibition Activity Data

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

Product Name: ProBiocin V™
Code Number: M14005
Lot Number: NC190905I
Test Request Number: 5739
CAS **#'s:** 68333-16-4 [AICS, Revised ICL] (or) 92128-79-5 [PICCS]
EINECS **#'s:** N/A (or) 295-777-8
INCI Name: Lactobacillus Ferment Lysate

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

QA Signature Monica Beltran

Date 11/8/2019

This is an Electronically Generated Document

ACTIVE MICRO TECHNOLOGIES

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

Inhibition Activity Data

Product Name: ProBiocin V™
Code Number: M14005
Test Request Number: 6193
CAS #'s: 68333-16-4 [AICS, Revised ICL] (or) 92128-79-5 [PICCS]
EINECS #'s: N/A
INCI Name: Lactobacillus Ferment Lysate

Organism (ATCC #)	Minimum Inhibitory Concentration (%)
Malassezia furfur #14521	1.0

QA Signature Monica Beltran

Date 03-30-2020

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(704) 276-7100 • Fax (704) 276-7101

Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

ProBiocin V™

Test Request Form

6199

Purpose

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

Study Dates

The study was started on October 9th, 2019 and was completed on December 13th, 2019.

Test Organisms

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

Neutralization:

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

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Test Method

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

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

Organisms					
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	2.5×10^6	3.5×10^6	3.2×10^6	2.0×10^5	1.3×10^5
Day 0	99.545%	>99.999%	99.846%	>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	5.2×10^6	5.5×10^5	2.6×10^6	3.1×10^5	4.3×10^5
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 pH 5 with 4% ProBiocin V™ inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

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

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Results & Discussion

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

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

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

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

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

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Phase	Ingredient	Supplier	%
I	Water	-	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^{0}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^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75^{0}C and mix for 15 minutes. Begin force cooling.

Phase III:
Add ingredients at 50^{0}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.

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Antimicrobial Efficacy (Challenge) Testing

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

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.

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Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

ProBiocin V™

Test Request Form

6192

Purpose

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

Study Dates

The study was started on January 29th, 2020 and was completed on March 27th, 2020.

Test Organisms

1. *Malassezia furfur:* ATCC #14521

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

Fifty grams of Generic Shampoo Formula with 4% ProBiocin V™ was weighed. The sample was inoculated with *Malassezia furfur*. The inoculum concentration for the microorganism 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.

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

Organism	**Sampling Interval**				
Inoculum Initial (CFU/ml)	Day 0	Day 7	Day 14	Day 21	Day 28
Malassezia furfur 5.6×10^5	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	Day 0	Day 7	Day 14	Day 21	Day 28
Malassezia furfur 2.5×10^5	N/A	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Shampoo Formula with 4% ProBiocin V™ 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. *Malassezia furfur* results are read up to 7 days.

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 Shampoo Formula with 4% ProBiocin V™. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

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.

Malassezia furfur was reduced by greater than 99.9% within 7 days of each challenge. By the end of each 28-day test period *Malassezia furfur* was reduced by 99.9% or greater.

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Antimicrobial Efficacy (Challenge) Testing

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

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.

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Time Kill Test

E2315

Assessment of Antimicrobial Activity Using a Time Kill Procedure

Product

ProBiocin V™

Test Request #:

5741

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 September 9th, 2019 and was completed on September 16th, 2019.

Test Organisms

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

Neutralization:

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

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Test Method

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

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

Organisms	Inoculum Concentration CFU/ml	Percentage of Reduction				
		30 seconds	1 minute	5 minute	10 minute	30 minutes
E.coli * ATCC# 8739	4.2×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
S.aureus ATCC# 6538	5.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
P.aeruginosa ATCC# 9027	2.3×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
B.subtilis ATCC# 6051	3.1×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
A.brasiliensis ATCC# 16404	3.0×10^5	99.9%	99.9%	99.9%	99.9%	99.9%
C.albicans ATCC# 10231	3.3×10^5	99.9%	99.9%	99.9%	99.9%	99.9%

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

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

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Results & Discussion

The results of this Time Kill Test determine the changes in population of aerobic microorganisms within a specified sampling time when tested against 4% ProBiocin V™ 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.

ACTIVE MICRO TECHNOLOGIES

Dermal and Ocular Irritation Tests

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Tradename: ProBiocin V™

Code: M14005

CAS #: 68333-16-4 [AICS, Revised ICL] (or) 92128-79-5 [PICCS]

Test Request Form #: 5592

Lot #: N190905I

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

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 **ProBiocin V™** 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% CO_2, and 95% relative humidity (RH). Tissue inserts were thoroughly washed and transferred to fresh plates with growth media. After post substance dosing incubation is complete, the cell viability test begins. Cell viability is measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2-yl*)], present in the cell mitochondria, into blue formazan salt that is measured after extraction from the tissue. The irritation potential of the test chemical is dictated by the reduction in tissue viability of exposed tissues compared to the negative control.

Under the conditions of this assay, the test article was considered to be **non-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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II. Materials

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

III. Test Assay

A. Test System

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

B. Negative Control

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

C. Positive Control

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

D. Data Interpretation Procedure

a. EpiDerm™

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

b. EpiOcular™

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

IV. Method

A. Tissue Conditioning

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

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B. Test Substance Exposure

a. EpiDerm™

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

b. EpiOcular™

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

C. Tissue Washing and Post Incubation

a. EpiDerm™

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

b. EpiOcular™

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

D. MTT Assay

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

V. Acceptance Criterion

A. Negative Control

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

B. Positive Control

a. EpiDerm™

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

b. EpiOcular™

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

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.

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B. Tissue Viability Assay
The results are summarized in Figures 1 and 2. In no case was the tissue viability ≤ 50% for EpiDerm™ or ≤ 60% for EpiOcular™ in the presence of the test substance. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited substantial loss of tissue viability and cell death.

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

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

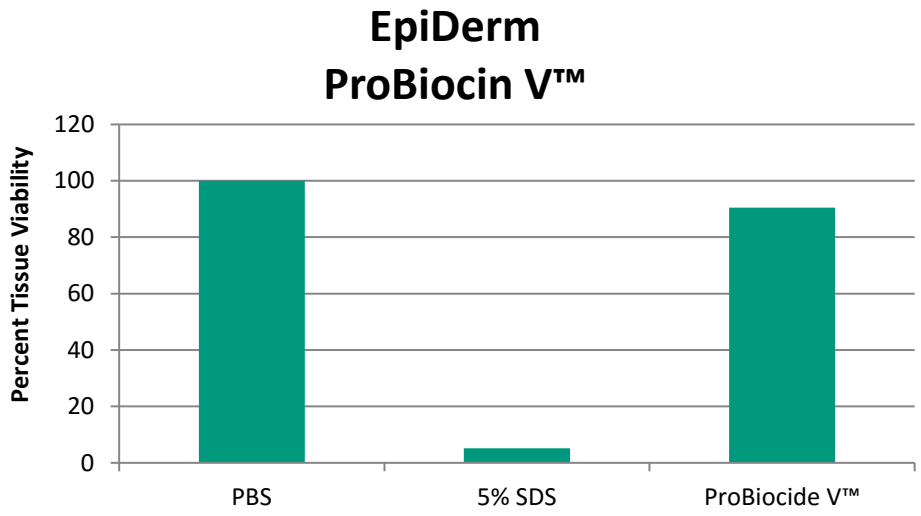

Figure 1: EpiDerm tissue viability

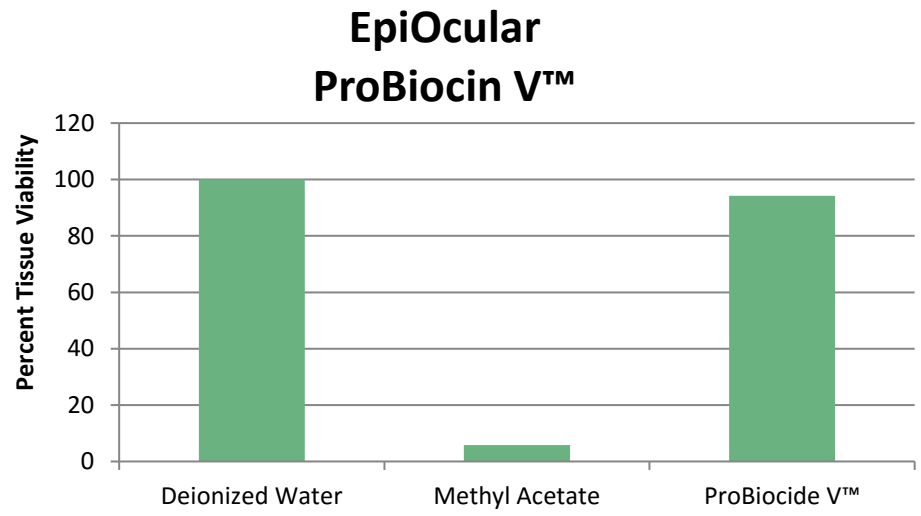

Figure 2: EpiOcular tissue viability

ACTIVE MICRO TECHNOLOGIES

OECD TG 442C: *In Chemico* Skin Sensitization

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Tradename: ProBiocin V™

Code: M14005

CAS #: 68333-16-4 [AICS, Revised ICL] (or) 92128-79-5 [PICCS]

Test Request Form #: 5591

Lot #: N190905I

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

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

Introduction

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

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

Assay Principle

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

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

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Materials

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

Methods

Solution Preparation:

- 0.667mM Cysteine Peptide in 100mM Phosphate Buffer (pH 7.5)
- 0.667mM Lysine Peptide in 100mM Ammonium Acetate Buffer (pH 10.2)
- 100mM Cinnamic Aldehyde in Acetonitrile
- 100mM* **ProBiocin V™** in Acetonitrile

*For mixtures and multi-constituent substances of known composition such as **ProBiocin V™,** 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
• 750µL Cysteine Peptide Solution (or 100mM Phosphate Buffer, pH 7.5, for Co-Elution Controls) • 200µL Acetonitrile • 50µL Test Chemical Solution (or Acetonitrile for Reference Controls)	• 750µL Lysine Peptide Solution (or 100mM Ammonium Acetate Buffer, pH 10.2, for Co-Elution Controls) • 250µL Test Chemical Solution (or Acetonitrile for Reference Controls)

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Calibration Curve:

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

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

HPLC Analysis:

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

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

Data and Reporting

Acceptance Criteria:

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

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

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Prediction Model:

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

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

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

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**
3.01	Minimal Reactivity	Non-sensitizer
3.00	Minimal Reactivity	Non-sensitizer
3.04	Minimal Reactivity	Non-sensitizer

Cysteine 1:10 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
3.03	Minimal Reactivity	Non-sensitizer
2.99	Minimal Reactivity	Non-sensitizer
2.97	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:

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

Based on HPLC-UV analysis of **ProBiocin V™ (M14005)** 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 **3.01%** causing minimal reactivity in the assay giving us the prediction of a non-sensitizer.

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OECD TG 442D: *In Vitro* Skin Sensitization

Tradename: ProBiocin V™

Code: M14005

CAS #: 68333-16-4 [AICS, Revised ICL] (or) 92128-79-5 [PICCS]

Test Request Form #: 5595

Lot #: N190905I

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

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

Introduction

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

This assay was conducted to determine skin sensitization potential of **ProBiocin V™** in accordance with the UN GHS.

Assay Principle

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

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

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

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Materials

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

Methods

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

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

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

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

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Data and Reporting

Acceptance Criteria:

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

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

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

Results

Compound	Classification	$EC_{1.5}$ (µM)	IC_{50}	I_{max}
Cinnamic aldehyde	Sensitizer	19	289.19 µM	32.08
DMSO	Non-Sensitizer	No Induction	243.24 µM	0.16
ProBiocin V™	Non-Sensitizer	No Induction	> 1000 µM	0.37

Table 1: Overview of KeratinoSens™ Assay Results

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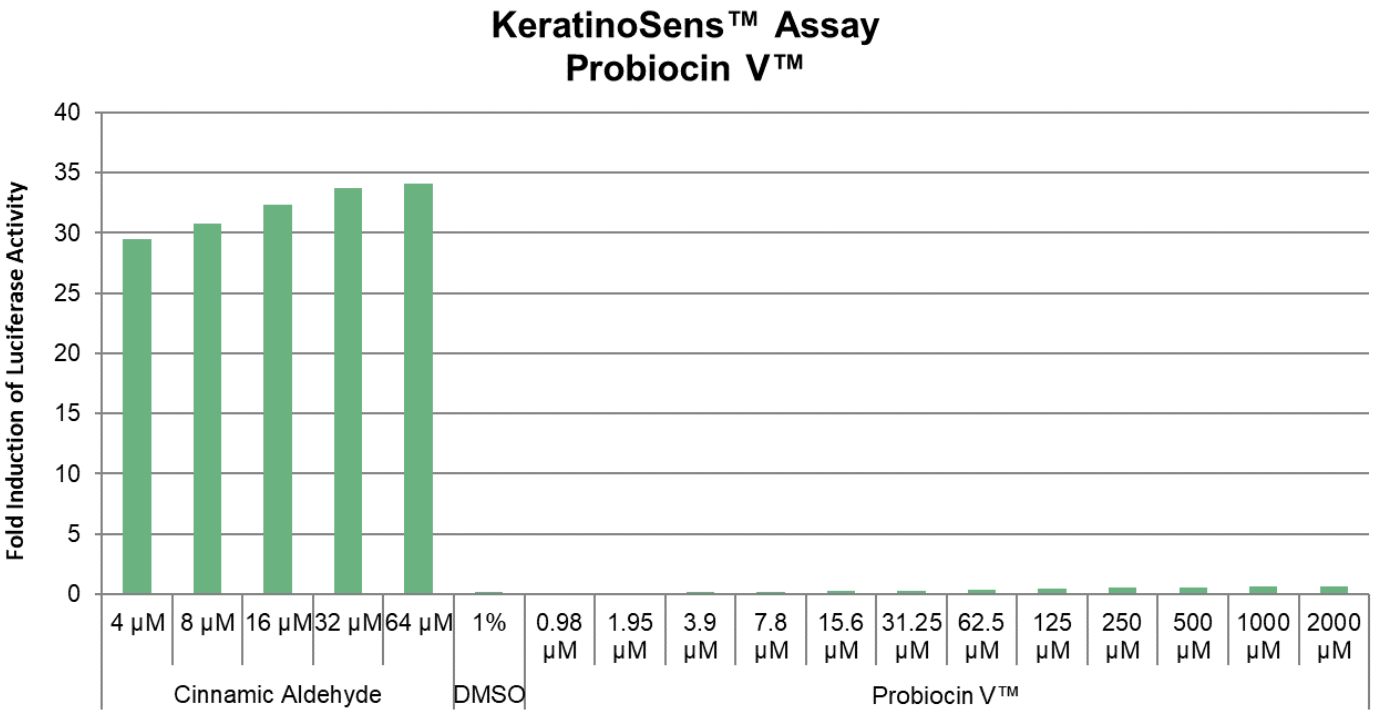

Figure 1: Fold Induction of Luciferase

Discussion

As shown in the results, **ProBiocin V™ (M14005)** 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 **ProBiocin V™** can be safely used in cosmetics and personal care products at typical use levels.

OECD 201 Freshwater Alga Growth Inhibition Test

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Tradename: ProBiocin V™

Code: M14005

CAS #: 68333-16-4 [AICS, Revised ICL] (or) 92128-79-5 [PICCS]

Test Request Form #: 5596

Lot #: N190905I

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 **ProBiocin V™** by exposing the exponentially growing test organism *Pseudokirchneriella subcapitata* to the test substance for 72 hours and measuring the growth and growth inhibition through cell counting against the control. The response is evaluated as a function of the exposure concentration in comparison with the average growth of replicate, unexposed control cultures.

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

Assay Principle

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

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

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

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Materials

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

Methods

Test Conditions

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

Measurement of Test Substance Concentrations

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

Observation

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

Test Condition Measurements

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

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Data and Reporting

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

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

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

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

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Results

General Information:

Name of new chemical substance	ProBiocin V™		
INCI Nomenclature	Lactobacillus Ferment Lysate		
CAS number	68333-16-4 [AICS, Revised ICL] (or) 92128-79-5 [PICCS]		
Formulation Method	Fermentation		
Molecular weight	2950.56 Da		
Purity of the new chemical substance used for the test (%)	100%		
Lot number of the new chemical substance used for the test	N190905I		
Names and contents of impurities	N/A		
Solubility in water	Soluble		
Properties at room temperature	Clear to Slightly Hazy Colorless to Yellow Liquid, Characteristic		
Stability	Stable Under Normal Conditions		
Solubility in solvents, etc.	**Solvent**	**Solubility**	**Stability in solvent**
	N/A	N/A	N/A

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OECD 201 Freshwater Alga Growth Inhibition Test

Test Materials and Methods:

Items			**Contents**
Test Organisms	Species		*Pseudokirchneriella subcapitata*
	Source		ATCC
	Reference substance (EC_{50})		3,5-dichlorophenol
Culture	Kind of Medium		Gorham's Medium for Algae
	Conditions (Temperature)		22°C ± 2°C
Test Conditions	Test Vessel		Glass
	Material Water	Kind	Deionized
		Hardness	250 mg/L
		pH	7.4
	Date of Exposure		10/07/2019
	Test Concentrations		200, 89.4, 42.3, 19.2, 7.8 mg/L
	Number of organisms		5 x 103-4 cells/ml
	Number of Replicates	Exposure Group	4
		Control Group	4
	Test Solution Volume		5 mL
	Vehicle	Use or Not	N/A
		Kind	N/A
		Concentration	N/A
		Number of Replicates	N/A
	Photoperiod		Continuous

Test Results:

Items		**Contents**
Toxicity Value	Percent Inhibition EC_{10} and EC_{20}	136.55 mg/L and 218.03 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 **ProBiocin V™** was determined to be 136.55 mg/L EC_{10} and 218.03 mg/L EC_{20}. The conditions of OECD guideline 201 for the validity of the test were adhered to, this product is not classified and therefore not harmful to aquatic organisms.

OECD 301B Ready Biodegradability Assay

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Tradename: ProBiocin V™

Code: M14005

CAS #: 68333-16-4 [AICS, Revised ICL] (or) 92128-79-5 [PICCS]

Test Request Form #: 5593

Lot #: N190905I

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

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

Introduction

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

Assay Principle

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

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

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

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

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

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

Materials

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

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- Stock solutions of test substances
  - When solubility of the substance exceeds 1 g/L, dissolve 1-10 g, as appropriate, of test or reference substance in water and make up to 1 liter. Otherwise, prepare stock solutions in mineral medium or add the chemical directly to the mineral medium, 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 CO_2-free air overnight to purge the system of carbon dioxide.
   d. Add the test material and reference compound, separately, as known volumes of stock solutions, to replicate flasks to yield concentrations, contributed by the added chemicals, of 10 – 20 mg DOC or TOC per liter. Leave some flasks without addition of chemicals as inoculum controls. Add poorly soluble test substances directly to the flasks on a weight or volume basis. Make up the volumes of suspensions in all flasks to 3 liters by the addition of mineral medium previously aerated with CO_2-free air.
   e. If required, use one flask to check the possible inhibitory effect of the test substance by adding both the test and reference substances at the same concentrations as present in the other flasks.
   f. If required, check whether the test substance is degraded abiotically by using a sterilized uninoculated solution of the chemical. Sterilize by the addition of a toxic substance at an appropriate concentration.
   g. If barium hydroxide is used, connect three absorption bottles, each containing 100 mL of 0.0125M barium hydroxide solution, in series to each 5-liter flask. The solution must be free of precipitated sulfate and carbonate and its strength must be determined immediately before use.
   h. If sodium hydroxide is used, connect two traps, the second acting as a control to demonstrate that all the carbon dioxide was absorbed in the first. Absorption bottles fitted with serum bottle closures are suitable. Add 200 mL 0.05M sodium hydroxide to each bottle. This is sufficient to absorb the total quantity of carbon dioxide evolved when the test substance is completely degraded.
   i. In a typical run, the following flasks are used:
      i. Flasks 1 & 2: containing test substance and inoculum (test suspension)
      ii. Flasks 3 & 4: containing only inoculum (inoculum blank)
      iii. Flask 5: containing reference compound and inoculum (procedure control)
      iv. Flask 6: containing test substance and sterilizing agent (abiotic sterile control)
      v. Flask 7: containing test substance, reference compound and inoculum (toxicity control)

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

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

Data and Reporting

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

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

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

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d. The percentage biodegradation is calculated from:

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

Or

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

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

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

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

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

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

Validity of Tests

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

ACTIVE MICRO TECHNOLOGIES

OECD 301B Ready Biodegradability Assay

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Data Sheet

Laboratory	Active Concepts Tissue Culture Laboratory		
Test Start Date	09/09/2019		
Test Substance	**Name**	ProBiocin V™	
	Stock Solution Concentration	2 g/L	
	Initial Concentration in Medium	20 mg/L	
Inoculum	**Source**	Activated Sludge	
	Treatment Given	Centrifugation	
	Pre-conditioning	N/A	
	Suspended Solids Concentration in Reaction Mixture	4 mg/L	
Reference Material	Sodium Benzoate	**Concentration**	20 mg/L
CO_2 Production and Degradability	**Method**	**$Ba(OH)_2$**	0.0125M
		NaOH	N/A
		Other	N/A
Total Contact Time	28 Days		
Total CO_2 Evolved Measurements	**Days**	2, 4, 11, 17, 23, 28	
Degradation Over Time	87% and 92**%** 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, **ProBiocin V™** achieved 89.5% biodegradation after 28 days of testing. The product met method requirements for the Readily Biodegradable classification.

ACTIVE MICRO TECHNOLOGIES

Phototoxicity Assay Analysis

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Tradename: ProBiocin V™

Code: M14005

CAS #: 68333-16-4 [AICS, Revised ICL] (or) 92128-79-5 [PICCS]

Test Request Form #: 5594

Lot #: N190905I

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

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

SUMMARY

In vitro phototoxicity irritation studies were conducted to evaluate whether **ProBiocin V™** 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% CO_2, and 95% relative humidity (RH). The following day, the appropriate tissue inserts were irradiated (UVA) for 60 minutes with 1.7 mW/cm^2 (=6 J/cm^2). After substance incubation, irradiation, and washing was completed, the cell viability test was conducted. Cell viability was measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2-yl*)], present in the cell mitochondria, into blue formazan salt that was measured after extraction from the tissue. The photoirritation potential of the test chemical was dictated by the reduction in tissue viability of UVA exposed tissues compared to non-UVA exposed tissues.

Under the conditions of this assay, the test article was considered to be **non-phototoxic** at concentrations of 0.5%, 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% CO_2 and 95% relative humidity
B. Equipment:	Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; UVA-vis Irradiation Equipment; UVA meter; Pipettes
C. Media/Buffers:	Dulbecco's Modified Eagle Medium (DMEM) based medium; Dulbecco's Phosphate-Buffered Saline (DPBS); sterile deionized H_2O
D. Preparation:	Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components
E. Tissue Culture Plates:	Falcon flat bottom 96-well, 24-well, and 6-well tissue culture plates
F. Reagents:	MTT (*3-4,5-dimethyl thiazole 2-yl*) (1.0mg/mL); Extraction Solution (Isopropanol); Chlorpromazine; Triton X-100 (1%)
G. Other:	Wash bottle; sterile disposable pipette tips; Parafilm; forceps

III. Test Assay

A. Test System

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

B. Negative Control

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

C. Positive Control

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

D. Data Interpretation Procedure

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

IV. Method

A. Tissue Conditioning

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

B. Test Substance Exposure

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

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C. Tissue Irradiation

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

D. Tissue Washing and Post Incubation

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

E. MTT Assay

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

V. Acceptance Criterion

A. Negative Control

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

B. Positive Control

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

C. Standard Deviation

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

VI. Results

A. Tissue Characteristics

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

B. Tissue Viability Assay

The results are summarized in Figure 1. Cell viability is calculated for each tissue as a percentage of the corresponding vehicle control either irradiated or non-irradiated. Tissue viability was not reduced by 20% in the presence of the test substance and UVA-irradiation at concentrations of 0.5%, 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.

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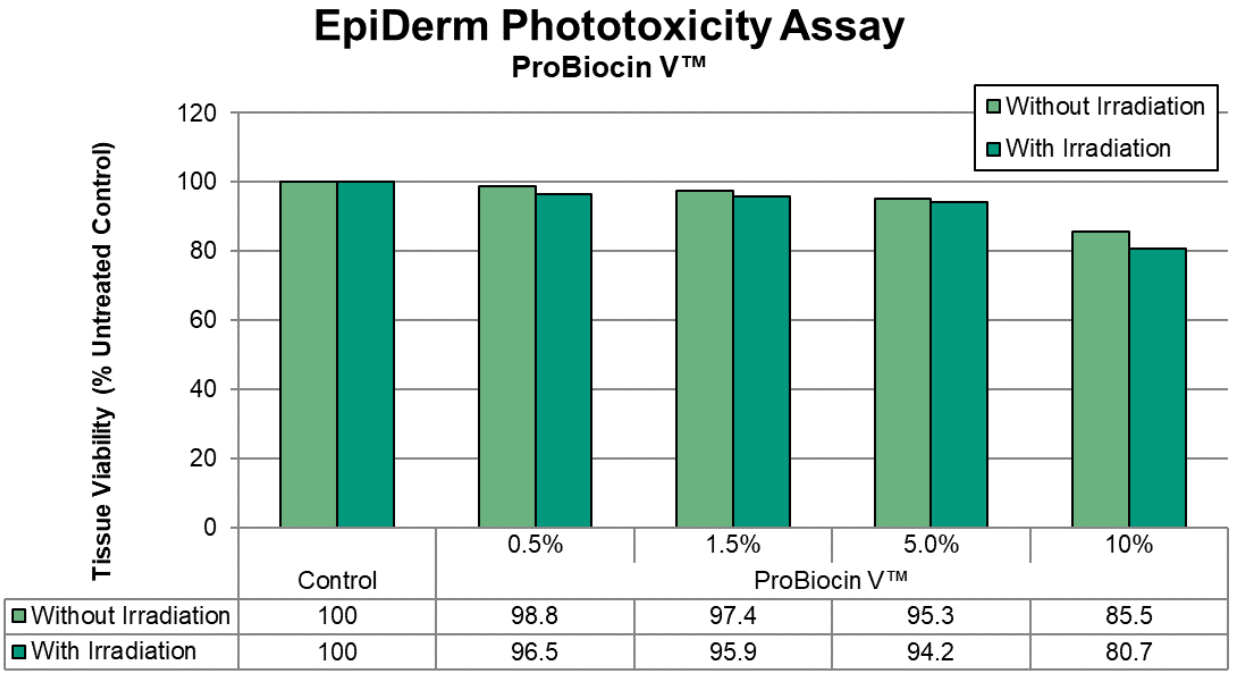
VII. Conclusion

Phototoxicity (photoirritation) is defined as an acute toxic response that is elicited after exposure of the skin to certain chemicals and subsequent exposure to light. Under the conditions of this assay, the test article substance was considered to be **non-phototoxic** at concentrations of 0.5%, 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 **ProBiocin V™** is not a photoirritant when used at the suggested use levels of 2.0% - 4.0%**.**



	Control	0.5%	1.5%	5.0%	10%
Without Irradiation	100	98.8	97.4	95.3	85.5
With Irradiation	100	96.5	95.9	94.2	80.7

Figure 1: EpiDerm Phototoxicity Graph

ACTIVE MICRO TECHNOLOGIES

Bacterial Reverse Mutation Test

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Test Article: ProBiocin V™
Code Number: M14005
CAS #: 68333-16-4 [AICS, Revised ICL] (or)
92128-79-5 [PICCS]

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: 5642

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 **ProBiocin V™** would cause mutagenic changes in the average number of reveratants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535 and tryptophan-dependent *Escherichia coli* strain WP2*uvr*A in the presence and absence of Aroclor-induced rat liver S9. This study was conducted to satisfy, in part, the Genotoxicity requirement of the International Organization for Standardization: Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

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

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

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

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

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I. Introduction

A. **Purpose**

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

II. Materials

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

III. Test System

A. Test System

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

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

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

B. Metabolic Activation

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

C. Preparation of Tester strains

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

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

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

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

IV. Method

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

V. Criteria for a Valid Test

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

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

VI. Results and Discussion

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

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

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

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

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

VII. Conclusion

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

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Appendix 2:

Bacterial Mutation Assay
Plate Incorporation Assay Results

	Concentration µg per Plate	*TA98*		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	20	22	21
	1500	19	15	17
	500	30	23	27
	150	18	30	24
	50	31	22	27
	15	18	20	19
	5.0	22	21	22
	1.5	25	16	21
Test Solution w/o S9	5000	20	22	21
	1500	35	32	34
	500	33	12	23
	150	20	20	20
	50	36	23	30
	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

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	Concentration µg per Plate	***TA100***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	156	188	172
	1500	124	144	134
	500	212	141	176
	150	110	135	123
	50	180	168	174
	15	160	188	174
	5.0	156	300	228
	1.5	172	136	154
Test Solution w/o S9	5000	112	125	119
	1500	114	212	163
	500	135	140	138
	150	111	163	137
	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

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	Concentration µg per Plate	***TA1537***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	21	9	15
	1500	36	11	24
	500	7	14	10
	150	11	6	9
	50	12	8	10
	15	13	8	11
	5.0	8	14	11
	1.5	13	8	11
Test Solution w/o S9	5000	21	20	21
	1500	19	10	15
	500	10	11	11
	150	4	8	6
	50	25	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

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	Concentration µg per Plate	***TA1535***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	36	13	25
	1500	13	7	10
	500	26	21	24
	150	20	15	16
	50	22	20	21
	15	24	11	18
	5.0	22	32	27
	1.5	16	14	15
Test Solution w/o S9	5000	88	82	85
	1500	72	95	84
	500	45	80	63
	150	84	80	81
	50	12	8	10
	15	12	12	12
	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

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	Concentration µg per Plate	***WP2uvrA***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	16	45	31
	1500	13	39	26
	500	25	40	33
	150	56	25	41
	50	16	10	13
	15	49	44	47
	5.0	35	55	45
	1.5	50	65	58
Test Solution w/o S9	5000	26	65	46
	1500	40	51	46
	500	25	32	29
	150	12	16	14
	50	33	30	32
	15	13	26	20
	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

ACTIVE MICRO TECHNOLOGIES

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Certificate of Origin

ProBiocin V™
Code: M14005

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-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.

SECTION 1. IDENTIFICATION

Product Name/Identifier	ProBiocin V™
Product Code	M14005
Recommended Use	Topical Cosmetic Use; Antimicrobial
Restrictions on Use	None
Supplier/Manufacturing Site	Active Micro Technologies, LLC
Address	107 Technology Drive Lincolnton, NC 28092, USA
Telephone No. (24hrs)	1-704-276-7100
Fax No.	1-704-276-7101
Emergency Telephone #	1-704-276-7100 (Mon-Fri: 8:00AM – 5:00PM EST)

SECTION 2. HAZARD(S) IDENTIFICATION

Classification:

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

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

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

Labeling Elements:

Pictograph: No hazard symbol expected

Hazard statements/Signal Word: Not applicable

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

Other hazards which do not result in classification:

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

Generic name:

Chemical Family: Ferment

Description: Substance

Substance	CAS Numbers	EC Numbers	Percentage
Lactobacillus Ferment Lysate	68333-16-4 (or) 92128-79-5	N/A (or) 295-777-8	100.00%

Formula: Not applicable

SECTION 4. FIRST-AID MEASURES

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

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

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

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

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

SECTION 5. FIRE-FIGHTING MEASURES

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

SECTION 6. ACCIDENTAL RELEASE MEASURES

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

SECTION 7. HANDLING AND STORAGE

Handling

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

Storage

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

SECTION 8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Precautionary statements: Ensure adequate ventilation

Control parameters

Occupational exposure Limits:

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

Personal Protective Equipment:

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

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

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

Solids (1g/1hr/105°C):	20.0 – 25.0%
pH (Direct):	4.0 - 11.0
Heavy Metals:	< 20 ppm
Lead:	< 10 ppm
Arsenic:	< 2 ppm
Cadmium:	< 1 ppm
Bacteriocins (HPLC):	5.00 – 10.00%
Minimum Inhibitory Concentration	
Organism (ATCC#):	
E. coli (#8739):	0.25 – 1.00%
S. aureus (#6538):	0.25 – 1.00%
P. aeruginosa (#9027):	0.25 – 1.00%
C. albicans (#10231):	0.25 – 1.00%
A. brasiliensis (#16404):	0.25 – 1.00%
Specific Gravity:	0.994
Vapor density:	Not applicable
Boiling Point:	100°C
Freezing Point:	0°C
Melting point:	Not applicable
Flash point:	> 200°F
Oxidizing properties:	Non oxidizing material according to EC criteria.
Solubility:	
In water:	Soluble
In organic solvents:	Not determined
Log P:	Not determined

SECTION 10. STABILITY AND REACTIVITY

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

Hazardous reactions: None known

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

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

Hazardous decomposition products: None known

SECTION 11. TOXICOLOGICAL INFORMATION

Ingestion: Not Determined
Dermal: Non-Irritant (Dermal Irritection Model)
Ocular: Non-Irritant (Ocular Irritection Model)
Inhalation: Not Determined

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

Sensitization: Non-Primary Irritant & Non-Primary Sensitizers; Will not cause allergic contact dermatitis (In Chemico Skin Sensitization Direct Peptide Reactivity Assay & In Vitro Skin Sensitization ARE-Nrf2 Luciferase Test Method)

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

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

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

Specific effects:

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

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

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

SECTION 12. ECOLOGICAL INFORMATION

Ecotoxicity
Effects on the aquatic environment: EC_{10} (Freshwater Alga): 136.55 mg/L - Not harmful to aquatic organisms
EC_{20} (Freshwater Alga): 218.03 mg/L - Not harmful to aquatic organisms

Biodegradability:
Persistence: Readily Biodegradable (89.5% biodegradation after 28 days of testing)

Bioaccumulation:
Octanol / water partition coefficient: Not Determined

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

Other Adverse Effects: None known

SECTION 13. DISPOSAL CONSIDERATIONS

Residues from product

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

Contaminated packaging

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

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

SECTION 14. TRANSPORT INFORMATION

UN Number: None
UN Shipping Name: None

Transport Hazard Class: Not classified as dangerous for transport

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

Marine Pollutant: No

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

Special Precautions for User: None known

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

SECTION 15. REGULATORY INFORMATION

Labeling:

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

Further regulations

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

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

Other regulations:

EINECS inventory status:	Lactobacillus Ferment Lysate:	N/A (or) 295-777-8
TSCA inventory status:	Exempt	
AICS inventory status:	Not Listed: 92128-79-5 Listed: 68333-16-4	
Canadian (CEPA DSL) inventory status:	Not Listed: Lactobacillus Ferment Lysate (92128-79-5) Listed as Lactobacillus acidophilus (Revised ICL)	
Japan (MITI list):	Lactobacillus Ferment Lysate	
Korea:	Lactobacillus Ferment Lysate	
China inventory status:	Lactobacillus Ferment Lysate	
Philippines inventory status:	Not Listed: Lactobacillus Ferment Lysate (68333-16-4) Listed as: Lactobacillus fermentatae, lysate	

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

SECTION 16. OTHER INFORMATION

Prohibited uses: For specific uses, food industry, ask the manufacturer for more information.

Last Revision Date: 02/11/2020

Preparation Date: 03/31/2020

MSDS summary of changes
- Updated Precautionary Statement – Section 2 (Hazards Information) & Updated Recommend Storage Conditions – Section 7 (Handling & Storage)
- Updated Tradename – Section 1 (Identification)
- Updated Europe Basis for Classification – Section 2 (Hazards Information)

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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.

M14005-**ProBiocin V™**
Manufacturing Flow Chart

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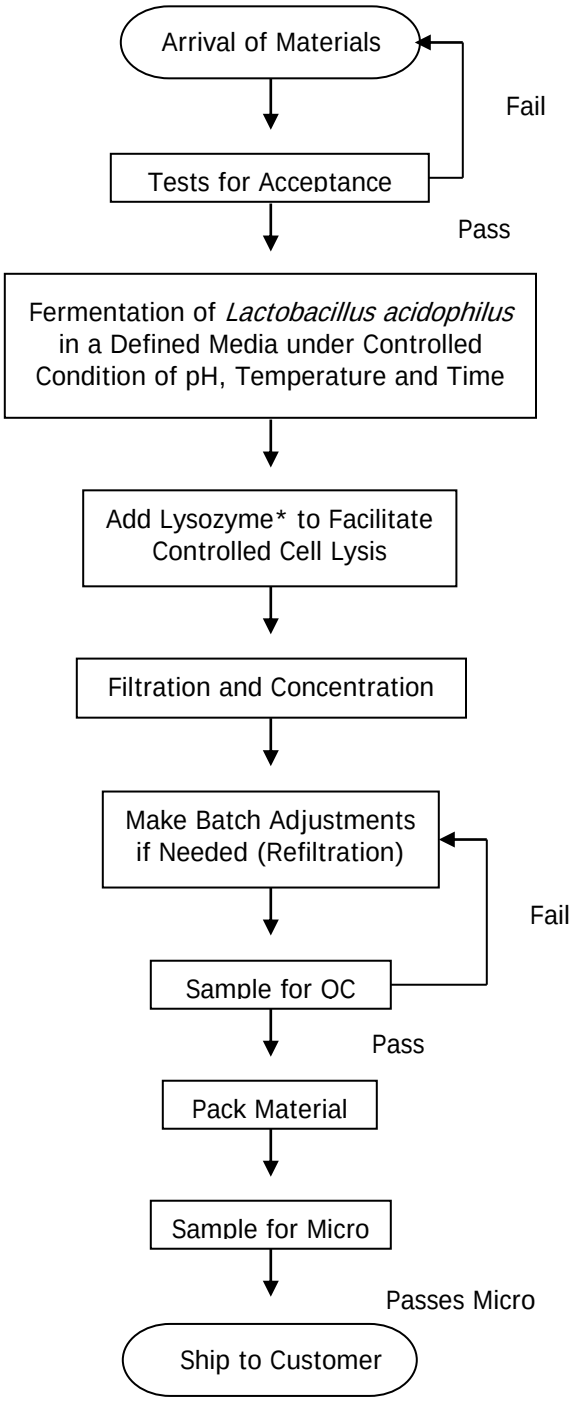

*Lysozyme is derived from Papaya (*Carica papaya*)

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ProBiocin V™
Certificate of Compliance

Code: M14005
INCI Name: Lactobacillus Ferment Lysate
INCI Status: Conforms
CAS #: 68333-16-4 [AICS, Revised ICL] (or) 92128-79-5 [PICCS]
EINECS #: N/A (or) 295-777-8

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)	Compliant
Mexico (COFEPRIS)	Compliant

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ProBiocin V™
Code: M14005

Attention must be paid to the use of ProBiocin V™ 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.

ProBiocin V™ 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 ProBiocin V™ is 2.00 – 4.00%.

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

ProBiocin V™ was tested using *in vitro* dermal and ocular irritation models. This product was found to be non-irritating in both models.

ProBiocin V™ is in compliance with the standardized set of rules developed and approved by the NPA (Natural Products Association).

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

(*) Carcinogenic, Mutagenic, toxic for Reproduction

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

Active Micro Technologies, LLC certifies that ProBiocin V™ does not contain any materials prohibited by Halal laws.

As of October 4, 2019, ProBiocin V™ 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 4, 2019.

ProBiocin V™ is REACH Compliant and free of the following:

- 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: ProBiocin V™ (M14005)

INCI Name: Lactobacillus Ferment Lysate

This is to certify that ProBiocin V™ is REACH compliant. Lactobacillus Ferment Lysate falls under the polymer exemption.

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

ACTIVE MICRO TECHNOLOGIES

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Date Issued: February 11, 2020

ALLERGEN DECLARATION

RE: *ProBiocin V™ (M14005)*

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

Heavy Metals Statement

107 Technology Drive • Lincolnton, NC 28092
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February 11, 2020

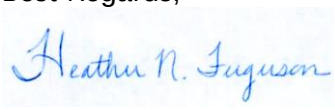
To Whom It May Concern,

This letter is to certify that ProBiocin V™ (M14005) has the following heavy metals profile:

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

**Please note: The above levels illustrate the Maximum Limits. Values for Chromium, Nickel, Antimony and Mercury do not appear on the Specification for ProBiocin V™.

Best Regards,



Tomorrow's Vision... *Today!* ®

Heather Ferguson |R&D Coordinator

107 Technology Drive | Lincolnton, NC 28092

Direct: 704.276.7083|Main: 704.276.7100|Fax: 704.276.7101

Email: hferguson@activeconceptsllc.com

www.activeconceptsllc.com

ATTESTATION OF CONFORMITY
- RAW MATERIALS -
COSMOS

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

ACTIVE MICRO TECHNOLOGIES LLC

107 Technology Drive
LINCOLNTON, NC 28092
UNITED STATES OF AMERICA

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

COSMOS (v3.0 – January 2019)

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

Issued in: L'Isle Jourdain,
the: 06/05/2020,

Emilie CHERHAL
ECOCERT Greenlife General Manager

Valid until: 31/12/2020

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

ATTESTATION OF CONFORMITY - COSMOS

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

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

Commercial name / INCI / Function	%PPAI	%CPAI	%NNI	%PeMo	Restriction	Approved since
AMTicide Coconut (M14003) *Lactobacillus (and) Cocos Nucifera (Coconut) Fruit Extract* Skin conditioning, Hair conditioning	0	100	0	0		01/01/2020
AMTicide® VAF (M14004) *Bacillus Ferment (and) Saccharomyces Ferment Filtrate* Skin Conditioning, Antifungal	0	100	0	0		01/01/2020
Arborcide OC (M15010) *Leuconostoc Ferment Filtrate* Skin conditioning, Antimicrobial	0	50	0	0		01/01/2020
Leucidal Advanced - Aloe (M15015) *Water (and) Leuconostoc/Aloe Barbadensis Leaf/Sorbus Aucuparia Fruit Ferment Filtrate* Moisturizing, Skin conditioning, Antimicrobial	0	18	0	0		01/01/2020
Leucidal Advanced - Rowan (M15018) *Water (and) Leuconostoc/Sorbus Aucuparia Fruit Ferment Filtrate* Emollient, Skin conditioning, Antimicrobial	0	16	0	0		01/01/2020

Valid until: 31/12/2020

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

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

ATTESTATION OF CONFORMITY - COSMOS

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

Commercial name / INCI / Function	%PPAI	%CPAI	%NNI	%PeMo	Restriction	Approved since
Leucidal Liquid (M15008) *Leuconostoc/Radish Root Ferment Filtrate* Moisturizing, Skin conditioning, Antimicrobial	0	52	0	0		01/01/2020
Leucidal Liquid AE LFHC (M15008LFHC) *Lactobacillus/Radish Root Ferment Filtrate* Skin conditioning, Antimicrobial	0	100	0	0		01/01/2020
Leucidal Liquid Complete (M15024) *Leuconostoc/Radish Root Ferment Filtrate (and) Lactobacillus (and) Cocos Nucifera (Coconut) Fruit Extract* Moisturizing, Skin conditioning, Antimicrobial	0	64	0	0		01/01/2020
Leucidal Liquid PT (M15021) *Lactobacillus Ferment* Skin conditioning, Antimicrobial	0	18	0	0		01/01/2020
Leucidal Liquid SF (M15019) *Lactobacillus Ferment* Moisturizing, Skin conditioning, Antimicrobial	0	10	0	0		01/01/2020
Leucidal Liquid SF (M15019RTZJV) *Leuconostoc/Radish Root Ferment Filtrate* Skin conditioning, Antimicrobial	0	10	0	0		01/01/2020
Leucidal SF Complete (M15025) *Lactobacillus Ferment (and) Lactobacillus (and) Cocos Nucifera (Coconut) Fruit Extract* Moisturizing, Skin conditioning, Antimicrobial	0	32,5	0	0		01/01/2020

Valid until: 31/12/2020

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

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

ATTESTATION OF CONFORMITY - COSMOS

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

Commercial name / INCI / Function	%PPAI	%CPAI	%NNI	%PeMo	Restriction	Approved since
Leucidal® Liquid J Max (M15030) *Leuconostoc/Radish Root Ferment Filtrate (and) Salix Alba (Willow) Bark Extract* Moisturization, Skin/Scalp Conditioning, Antimicrobial	20	30	0	0		06/05/2020
Leucidal® SF Max (M15019MAX) *Lactobacillus Ferment* Ferment / Skin Conditioning, Antimicrobial	0	25	0	0		01/01/2020
Myavert (M13001) *Lactoperoxidase (and) Glucose Oxidase* Skin conditioning, Hair conditioning, Antimicrobial	0,95	0,95	0	0		01/01/2020
PhytoCide Aspen Bark Extract Powder (M16002) *Populus Tremuloides Bark Extract* Skin conditioning, Antimicrobial	100	0	0	0		01/01/2020
PhytoCide Black Currant Powder (M16001) *Ribes Nigrum (Black Currant) Fruit Extract* Soothing, Skin conditioning, Antimicrobial	100	0	0	0		01/01/2020
PhytoCide Elderberry OS (M16003) *Sambucus Nigra Fruit Extract* Skin conditioning, Antimicrobial	100	0	0	0		01/01/2020
ProBiocin V™ (M14005) *Lactobacillus Ferment Lysate* Antimicrobial, Redness Reduction, Scalp Care	0	100	0	0		06/05/2020

Valid until: 31/12/2020

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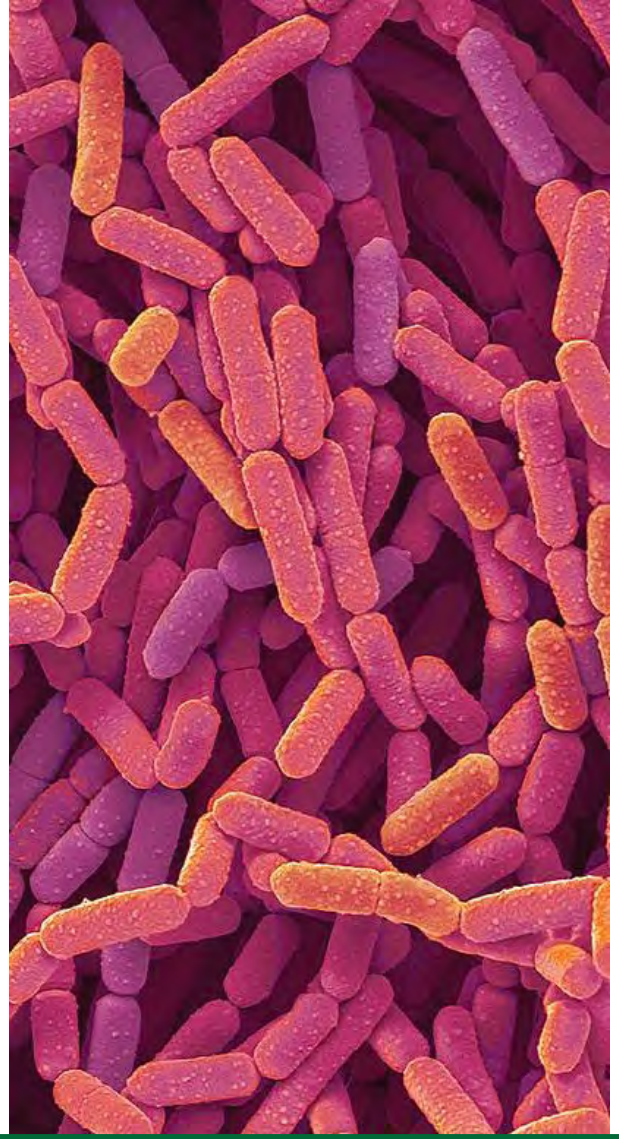
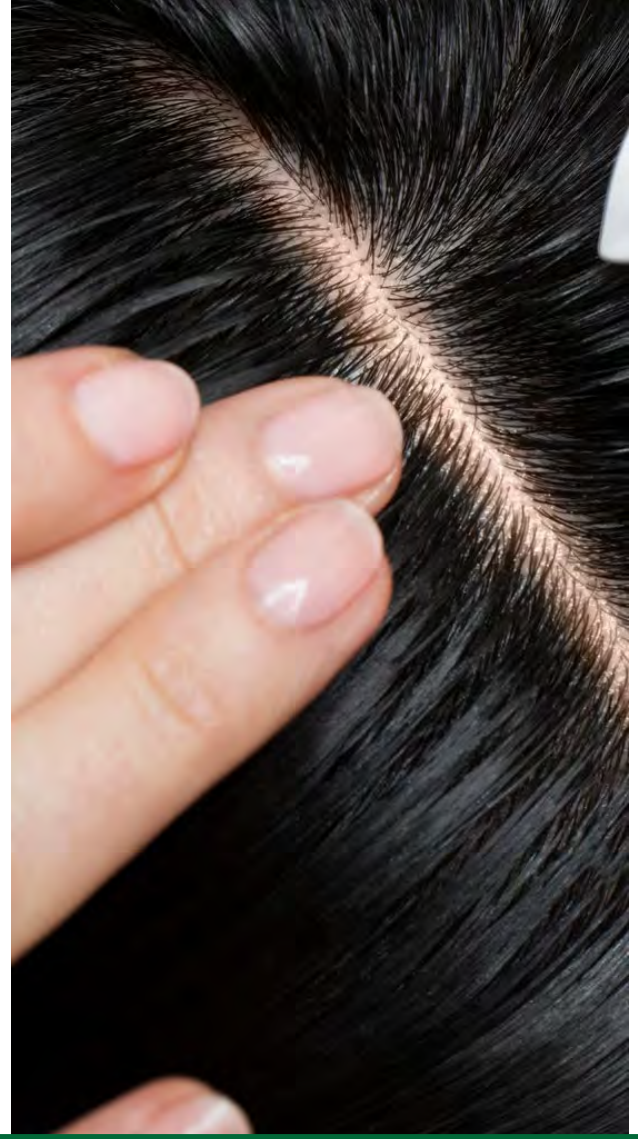




ACTIVE MICRO TECHNOLOGIES

ProBiocin V™

ProBiocin V™

Vegan, Globally Compliant, Antimicrobial

- **Product Code:** M14005
- **INCI Name:** Lactobacillus Ferment Lysate
- **Suggested Use Levels:** 2.00% - 4.00%
- **Suggested Applications:** Redness Reduction, Scalp Care, Antimicrobial
- **Solubility:** Water Soluble

ProBiocin V™

Multifunctional Ingredients – Effective and Marketable

- Derived from *Lactobacillus acidophilus,* a **probiotic bacterium** traditionally used to ferment milk into yogurt. Created by the fermentation of *Lactobacillus* in a defined growth medium, this specific manufacturing process yields a unique peptide with broad spectrum, antimicrobial and conditioning properties.

- **ProBiocin V™** – multifunctional balance
  - Antimicrobial peptide (bacteriocin) content provides antibacterial activity against pathogenic bacteria like *Malassezia spp.*
  - Implements optimal scalp care for increased moisturization and decreased erythema
  - Capable of reducing red areas of the face

- A **next generation active** capable of protecting formulation integrity while simultaneously providing brand differentiation and comprehensive care for the skin the scalp.

ProBiocin V™

Manufacturing Process

Prebiotic chicory oligosaccharide fructans are supplemented into *Lactobacillus acidophilus* growth media

Fermentation of *Lactobacillus acidophilus* in a defined media under controlled conditions

- Produces bacteriocins to reduce pathogens and promote a healthy skin and scalp

Once fermentation is complete, **lysozyme** is added to facilitate cell lysis and ensure release of bacteriocins

Material is filtered to remove undesired plant matter and bacteria

Multitasking Beauty

Multifunctional Cosmetics – Effective and Marketable

- **Result-driven** products with **characterized activity** cut down on the ingredient deck in formulations.
- Typical anti-aging products are losing momentum as **multifunctional** ingredients take center stage.
- Busy lifestyles of consumers opt for convenience products – beauty products with multiple benefits and the maximum value for amount spent.
- Sustainability and the conscious consumer
  - A rise in health consciousness among consumers push for ingredients for healthy, balanced lifestyles.
  - Natural **probiotics**, skin protection, and preventative actives with minimal environmental impact.

"Consumers are gravitating toward multi-functional products that allow them to get more benefits while saving them both time and money"

-Mintel[1]

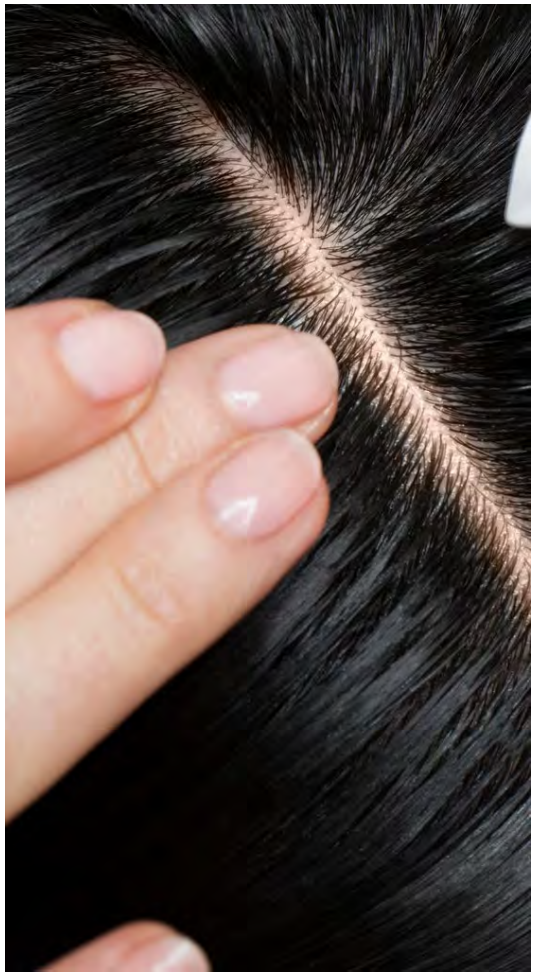
1. https://www.cosmeticsdesign.com/Article/2013/07/22/What-do-consumers-want-Mintel-reveals-US-facial-skin-care-trends.

Skin & Scalp Care

"Health is Wealth"

- Scalp care is a prevalent branch of hair care. A healthy scalp means healthy hair growth!
  - Dandruff has been shown to effect approximately 50% of the population, regardless of gender or ethnicity[1], driving consumer's interests in products that can restore balance to an "unhealthy" scalp.

- *Malassezia spp.* has been shown to play a key role in the pathogenesis of dandruff.
  - A study performed on a group of dandruff patients resulted in 84% of participants having a species of *Malassezia* present on the scalp[2].
  - Inspired by dandruff related irritation, research has driven product development to address *Malassezia* and balance the scalp microbiome.



1. Ranganathan, S., and T. Mukhopadhyay. "Dandruff: The most commercially exploited skin disease." Indian journal of dermatology 55.2 (2010): 130.
2. Rudramurthy, Shivaprakash M., et al. "Association of Malassezia species with dandruff." The Indian journal of medical research 139.3 (2014): 431.

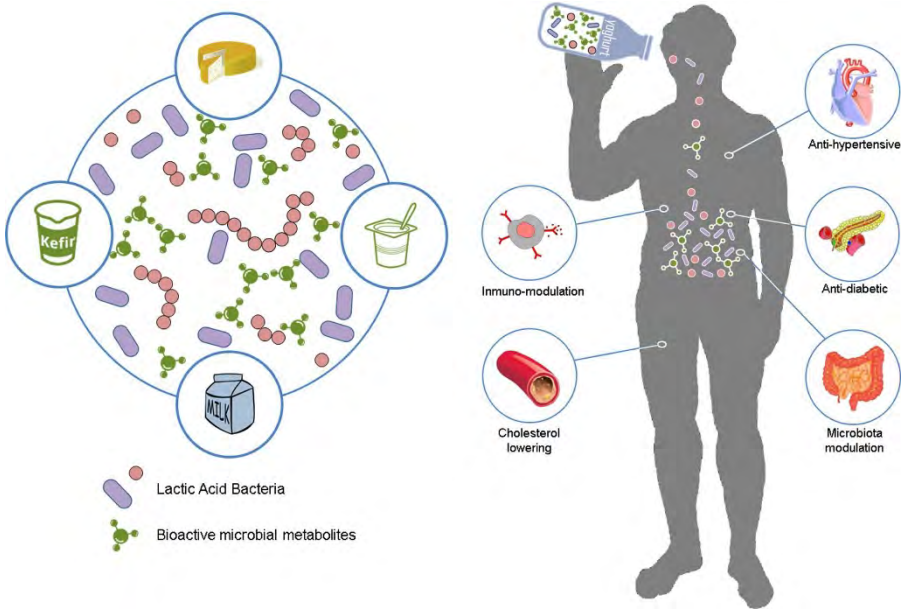
Probiotic Trend

The Rise of Probiotics in Wellness and Skin Care

- In recent years, awareness has been growing around the key role **probiotics** play in **digestive health**.
  - The global market for probiotic ingredients in 2017 was valued at **36.6 billion** USD and predicted to exceed **64 billion** USD by 2023[4].

- Oral **probiotic** supplements and **fermented** dairy products are well recognized for balancing and protecting the gut's **microflora** and supporting the body's **immune** system.

- Research is emerging to support the notion that **topical probiotics** are beneficial for skin.
  - The topical use of probiotics will confer similar protective balance to the skin, and therefore help keep skin strong, healthy and age resistant.



4. 2018 Wellness Trends, from Global Market Insights Inc.

Our Innovative Probiotic Approach

Bacterial Lysates for Probiotic Benefit

- **Probiotics** are defined by the Food and Agriculture Organization of the **World Health Organization** as **live microorganisms** that, when administered in adequate amounts, confer a health benefit to the host.

- Within cosmetics, there are **restrictions** placed on the microbial content of a product as indicated in the **US FDA'S Guidelines**. In order for probiotics to deliver the desired benefit they must be present in billions of colony forming units (CFU). This level of probiotic incorporated into a cosmetic product would **exceed the microbial limit** as designated by the FDA.

- Active Micro Technologies has taken an **innovative approach** through the use of **bacterial lysates** for probiotic benefit. The bacterial lysate has all the beneficial active components of live bacteria, **without the live bacteria**.

- Through the specific technique of supplementing growth media with prebiotics to stimulate the probiotic attributes of *Lactobacillus acidophilus*, Active Micro Technologies is able to **isolate** and **extract** novel postbiotic active compounds for a **powerful probiotic active**!

Veganism Trend

Moving Forward

- Veganism is a popular trend in the cosmetic industry. As consumers are making lifestyle choices that make them feel healthier, the same logic applies to the products they use.

- Although you do not have to be vegan to use vegan products, consumers are beginning to pay more attention to what goes into the products they use and how these products will benefit them long-term.

- While shifting towards animal alternatives and away from ethical concerns, sacrificing the quality of ingredients is not an option when it comes to treating common cosmetic concerns such as moisturization and redness.

Preservative Systems

Choosing the Right One

- Consumer concerns often pertain to the use of synthetic preservatives such as parabens, formaldehyde donors and phenoxyethanol in their products[5]. Consumers often perceive traditional preservative systems as being associated with sensitization[6].

- Traditional preservatives have been shown to be detrimental to both commensal and pathogenic bacteria living on the skin.

- **ProBiocin V™** is a technology able to diminish these concerns, while providing efficacious benefits against scalp irritation. These peptides are also capable of providing broad spectrum antimicrobial protection in order to maintain the integrity of the formulation.

5. Lee, Eun-Young, et al. “A Study of Influencing Factors for Sensory Irritation Due to Preservatives of Cosmetics.” Journal of the Society of Cosmetic Scientists of Korea, Society of Cosmetic Scientists of Korea, www.koreascience.or.kr/article/JAKO200616419600251.page.
6. Kabara, Jon J., and Donald S. Orth. Preservative-Free and Self-Preserving Cosmetics and Drugs: Principles and Practices. Marcel Dekker, 1997.

ProBiocin V™

Available Efficacy Data

- ***In-vivo* Efficacy Studies**
  - VISIA® Analysis
  - Scalp Care Study

- ***In-vitro* Efficacy Studies**
  - Minimum Inhibition Concentration (MIC) Data
    - *E. Coli, P. aeruginosa, S. aureus, A. brasiliensis, C. albicans, M. furfur.*
    - *M. furfur* MIC Comparison Data
  - Cream Formula Challenge Test at 4.0% use level at pH 3, 5, & 7
  - Shampoo Formula Challenge Test at 4.0%
  - Time Kill Test

- **Safety Information**
  - *in-vitro* Dermal and Ocular Irritation Tests
  - Bacterial Reverse Mutation Test
  - Phototoxicity Test
  - OECD 442D TG in-vitro Skin Sensitization
  - OECD 201 Freshwater Alga Growth Inhibition Test
  - OECD 301B Ready Biogradability Assay
  - Allergen Statement

ProBiocin V™

VISIA® Complexion Analysis: Average Red Area Feature Counts

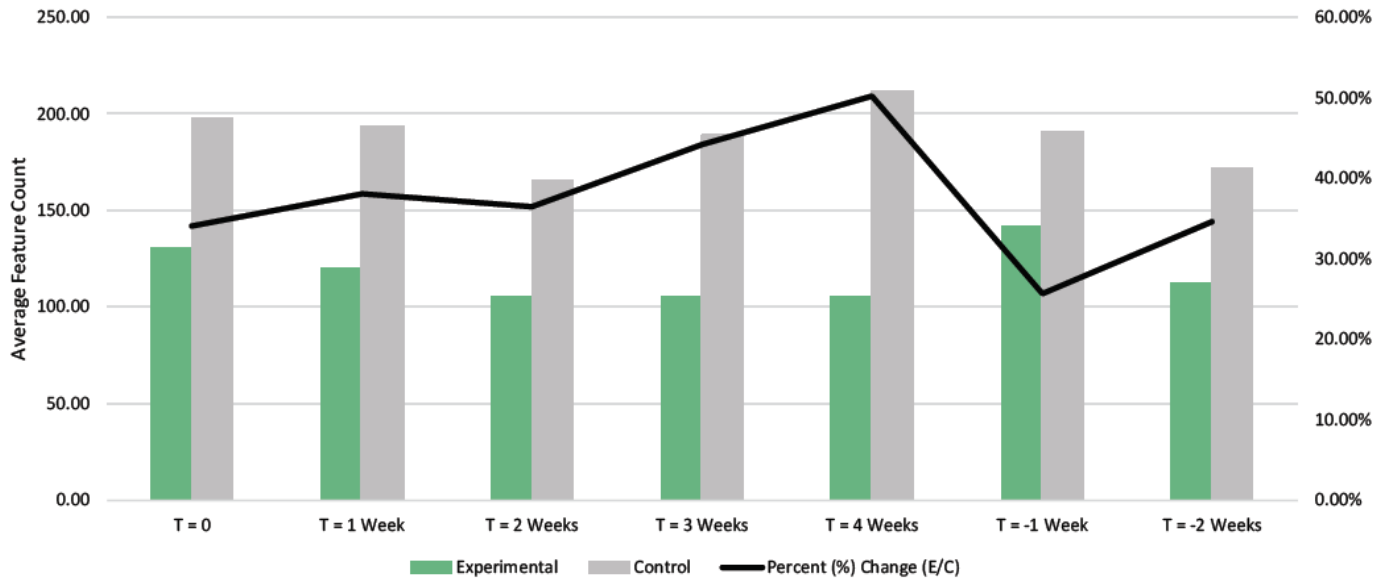


Figure 1: Average red area feature count

Protocol

- An in-vivo VISIA® Complexion Analysis study was conducted using 10 M/F participants between the ages of 23-55
- Each participant was instructed to apply 2.0 mg of lotion to their entire face twice a day for a four week period. Participants were instructed to continue their usual skin care routine and to apply the lotion once their everyday skin care routine is finished
- Half of the participant population used 4% **ProBiocin™** Cetaphil Daily Facial Moisturizer for all skin types, while the other half used the Cetaphil Daily Facial Moisturizer alone as a control
- Feature counts provide a count of the number of discrete instances of the feature being evaluated
- **ProBiocin™ V** in base lotion displayed a reduced number of red areas when compared to the control

ProBiocin V™

VISIA® Complexion Analysis: Average Red Area Feature Counts

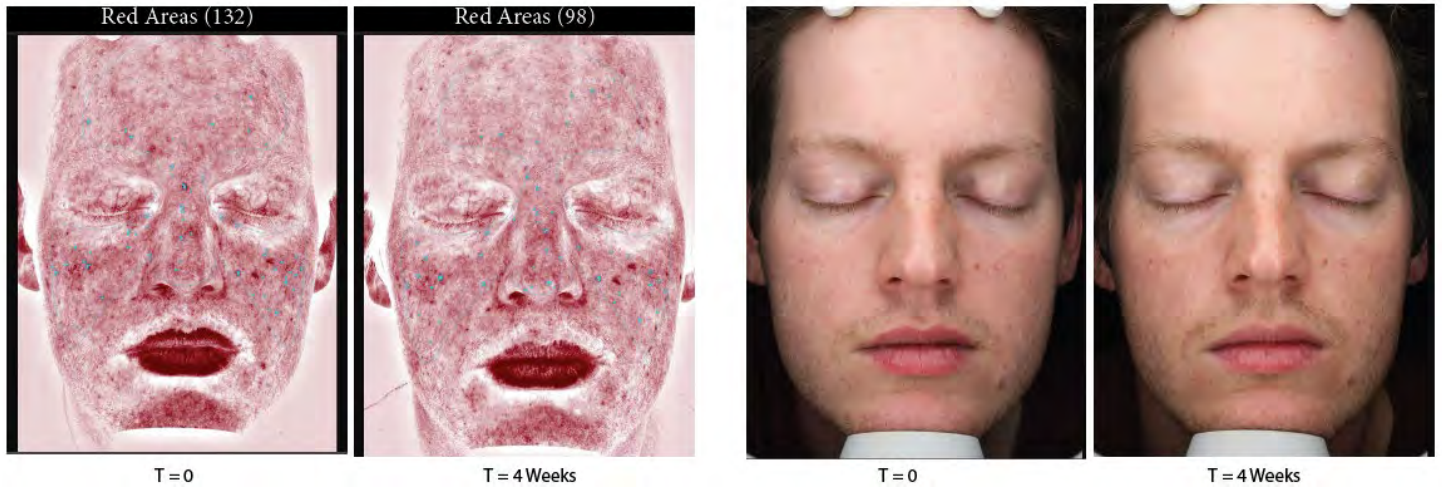


Figure 2: Panelist #3 treated with 4.0% **ProBiocin™ V** in Base Lotion displays a reduction (25.8%) in feature counts for red areas from beginning of treatment (T=0) to T=4 Weeks via VISIA Image Analysis. Images on the left are panelist #3 with image enhancement, through VISIA, which provides higher visualization of feature changes. Images on the right are natural photos of panelist #3.

Figure 3: Panelist #3 treated with 4.0% **ProBiocin™ V** in Base Lotion displays a increase (31.6%) in feature counts for red areas (due to the halted use of product) from 4 weeks to -2 weeks (regression) via VISIA Image Analysis. Images on the left are panelist #3 with image enhancement, through VISIA, which provides higher visualization of feature changes. Images on the right are natural photos of panelist #3.

ProBiocin V™

Scalp Care Study

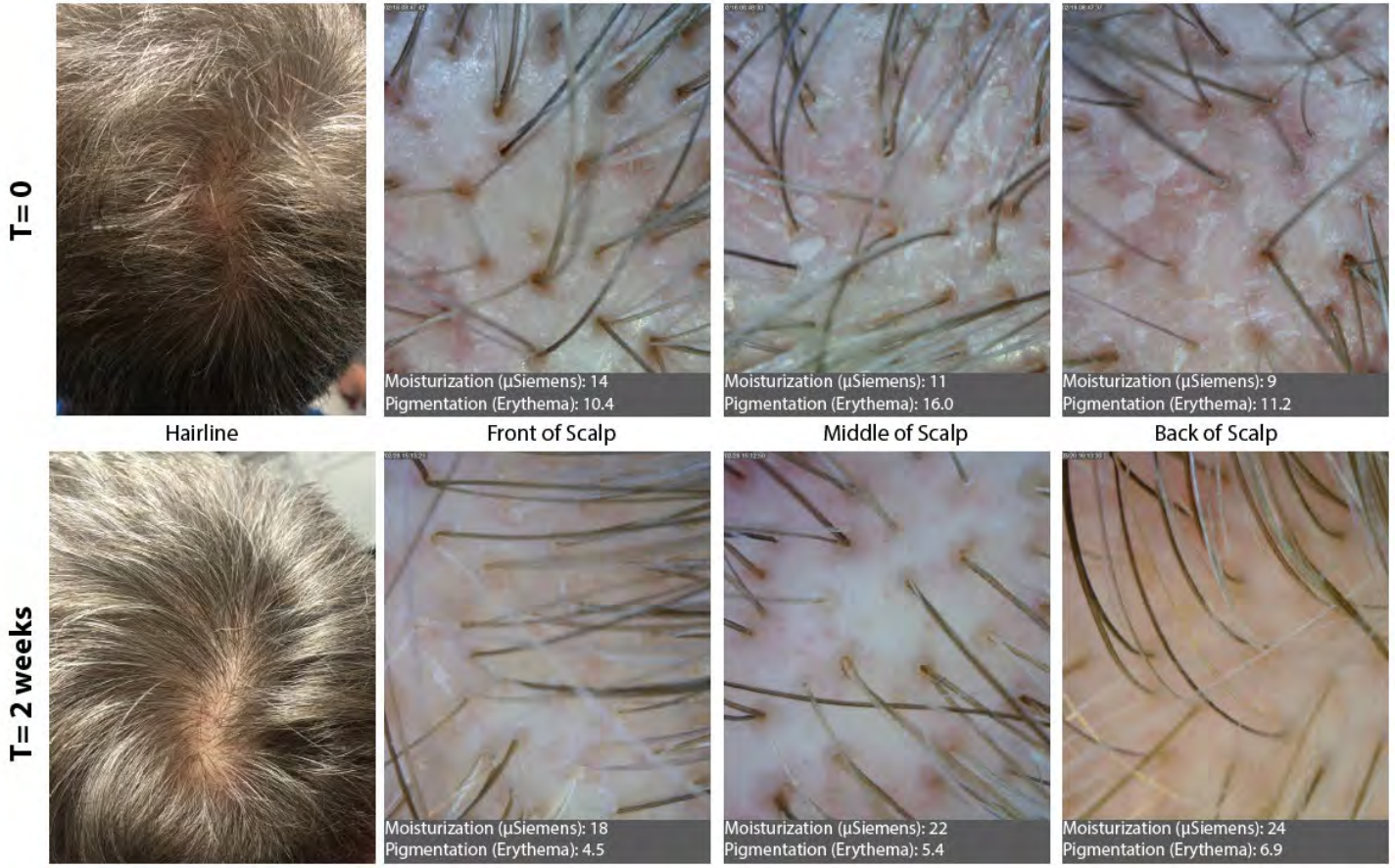


Figure 4: Images of Panelist #4 treated with 4.0% **ProBiocin V™** in Base Shampoo and Conditioner display an increase in overall scalp moisturization (front, middle, back averages) by 88.26% and decrease in overall scalp erythema (front, middle, back averages) by 55.31% from beginning of treatment (T=0) to T=2 Weeks via DermaLab analysis.

Protocol

- An in-vivo Scalp Care Study was conducted using 10 M/F participants between the ages of 23-55
- Panelist's heads were treated with either the base shampoo and conditioner or the experimental containing 4.0% **ProBiocin V™** in the base shampoo and base conditioner
- After the application and rinse of the experimental and control applications, each panelist's hair was blown dry using a round brush
- Each panelist had his or her head washed three times per week for a two week period
- After the third wash of the week, panelists had scalp moisturization and pigmentation values recorded

ProBiocin V™

Minimum Inhibition Concentration (MIC) Data

- The ability of **ProBiocin V™** to inhibit the growth of a variety of bacteria and fungi was determined using the Minimum Inhibitory Concentration (MIC) test. The results are illustrated in Figure 5, showing that this material provides broad spectrum antimicrobial protection.

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

Figure 5: MIC data for **ProBiocin V™**.

- The ability of **ProBiocin V™** to inhibit the growth of Malasezzia furfur, in comparison to industry leading anti-fungals, was determined using the Minimum Inhibitory Concentration (MIC) test. The results are illustrated in Figure 6, showing that **ProBiocin V™** provides comparable anti-fungal protection to Piroctone Olamine, while out performing Climbazole.

Product Name	MIC (%) to Inhibit *M. furfur*
ProBiocin V™	1.00
Climbazole	2.00
Piroctone Olamine	1.00
Zinc Pyrithione	0.01

Figure 6: *M. furfur* MIC comparison data.

ProBiocin V™

Challenge Test

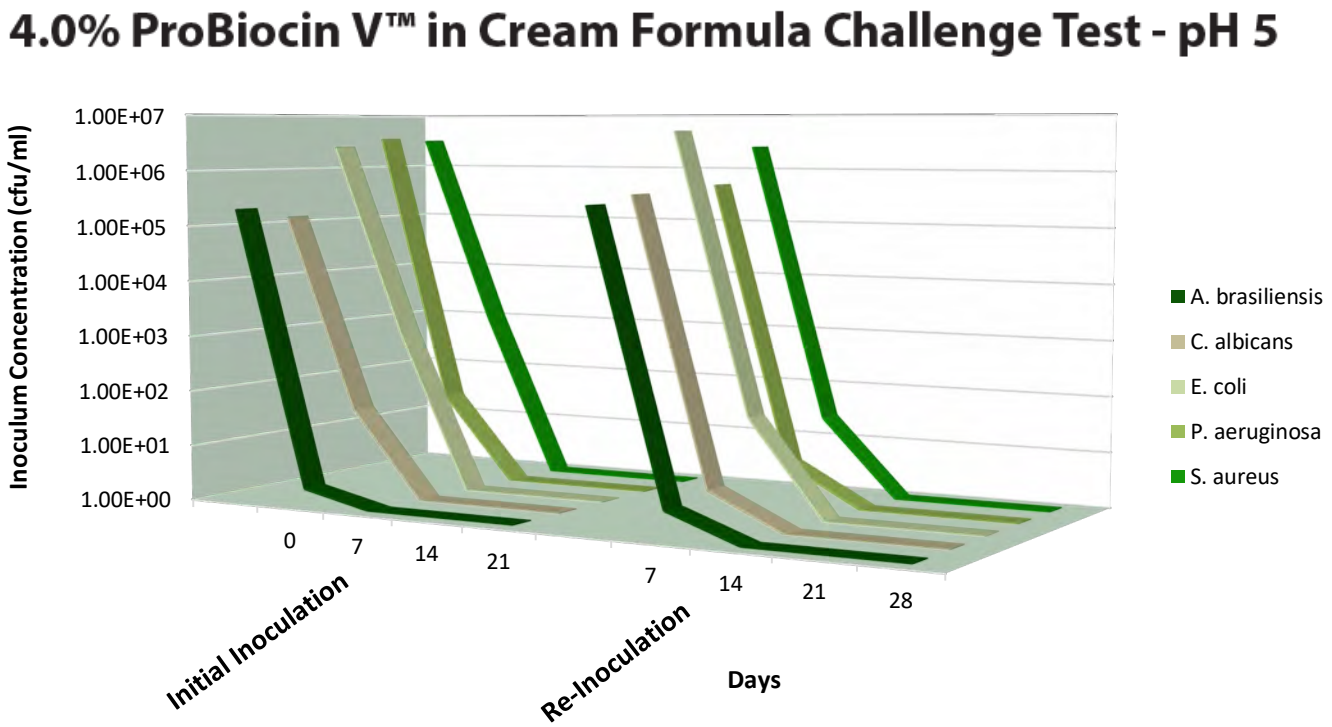


Figure 7: Challenge Test Results for Generic Cream Formula pH 5 with 4.0% **ProBiocin V™** inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

Protocol

- Samples were inoculated with microorganisms
  - *E. Coli, P. aeruginosa, S. aureus, A. brasiliensis, C. albicans.*
- During the first 28-day incubation period, samples were periodically collected and tested for the presence of these microorganisms.
- Following this initial 28 days of incubation, the cream samples were then re-inoculated with the cultures and sampled over an additional 28-day period.

M14005 ProBiocin V™ demonstrated successful antimicrobial protection

ProBiocin V™

Challenge Test

4.0% ProBiocin™ V in Shampoo Formula Challenge Test

Organism	Sampling Interval				
Inoculum Initial (CFU/ml)	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Malassezia furfur 5.6 x 10^5	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Re-innoculation (CFU/ml)	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Malassezia furfur 2.5 x 10^5	N/A	>99.999%	>99.999%	>99.999%	>99.999%

Figure 8: Challenge Test Results for Generic Shampoo Formula with 4.0% **ProBiocin V™** inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

Protocol

- Samples were inoculated with *Malassezia furfur.*
- During the first 28-day incubation period, samples were periodically collected and tested for the presence of these microorganisms.
- Following this initial 28 days of incubation, the cream samples were then re-inoculated with the cultures and sampled over an additional 28-day period.

M14005 ProBiocin V™ demonstrated successful antimicrobial protection

ProBiocin V™

Marketable and Effective

- **Moisturizing, Redness Reduction, Scalp Care**

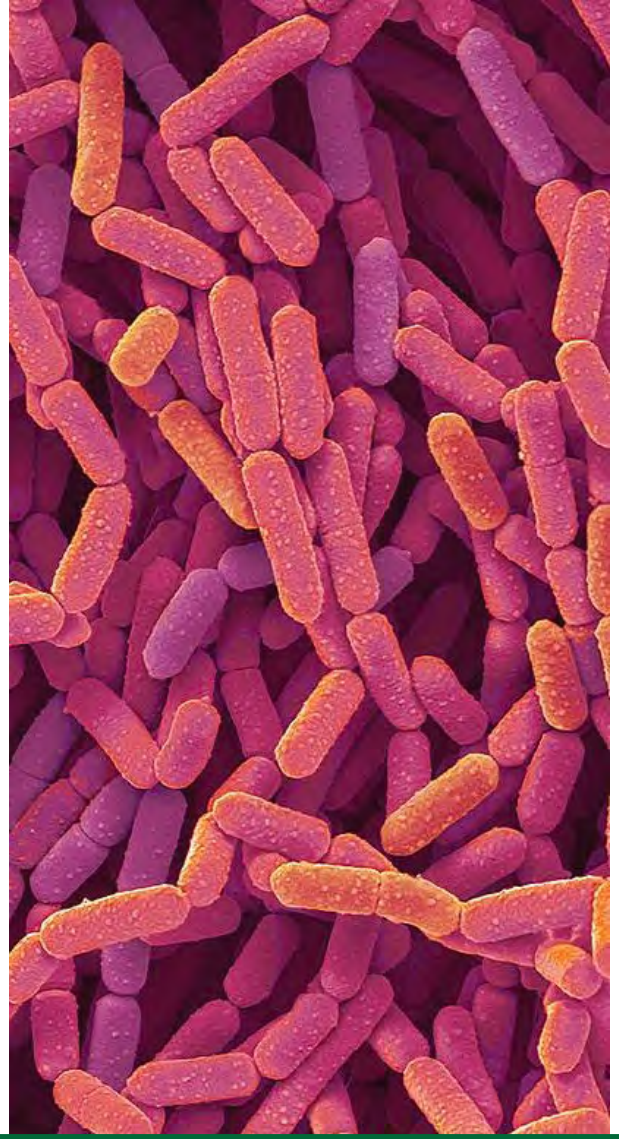
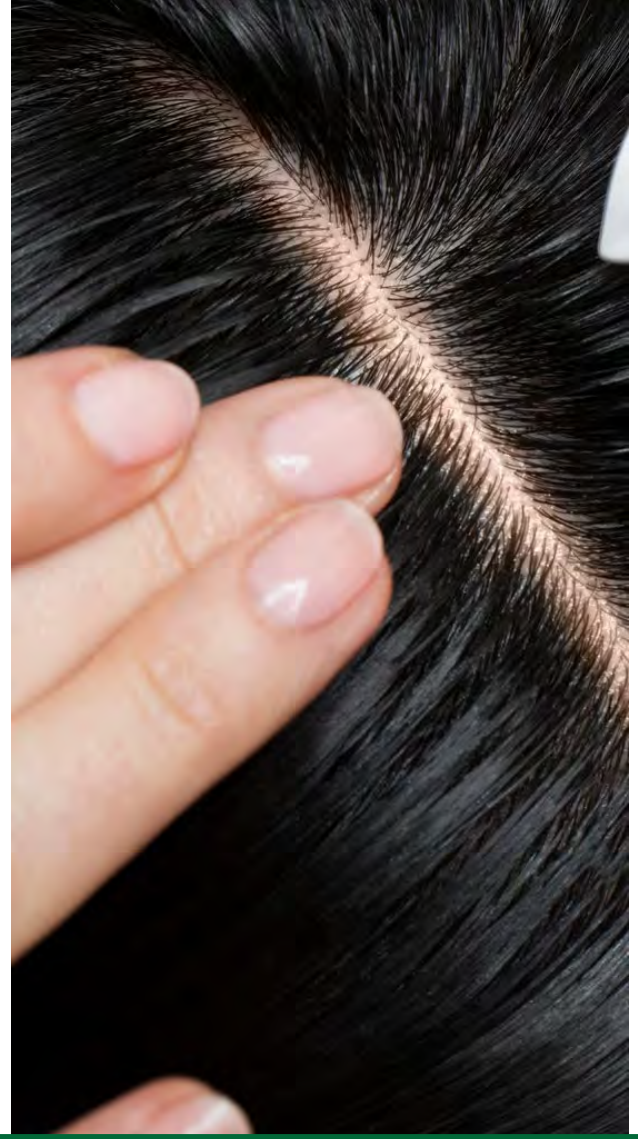
- Natural, multifunctional product – fits any application

- Effective and marketable
  - Multifunctional active
  - Probiotic & prebiotic provenance
  - Deployed postbiotic technology
  - COSMOS compliant
  - Vegan compliant

ProBiocin V™

Vegan, Globally Compliant, Antimicrobial

- **Product Code:** M14005
- **INCI Name:** Lactobacillus Ferment Lysate
- **Suggested Use Levels:** 2.00% - 4.00%
- **Suggested Applications:** Redness Reduction, Scalp Care, Antimicrobial
- **Solubility:** Water Soluble


Active Micro Technologies, LLC
Lincolnton, NC. USA
www. activemicrotechnologies.com
Office: +1 704 276 7100
info@activemicrotechnologies.com

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