

BACKGROUND

Over the past several decades there has been growing public pressure, increasingly strict chemical regulations, preservative sensitization issues, and the potential for developing microbial resistance to the chemical preservative products typically used in cosmetic and personal care formulations. These factors have resulted in numerous methods of preservation being pulled from the marketplace, despite being the products of choice at one time. To offer a solution to this preservation paradigm, **Active Micro Technologies (AMT)** has developed a line of products based on naturally occurring compounds that provide active cosmetic properties, but by their very nature are also capable of providing product preservation. This antimicrobial capability is due to natural mechanisms developed by plants and microorganisms by which they protect themselves from their environment and other competing organisms.

SCIENCE

Leucidal® Liquid is based on an antimicrobial peptide originally derived from the lactic acid bacteria, *Leuconostoc kimchii*. *L. kimchii* is one of 15 species of microorganisms that make up the mixed culture used for producing the Korean dietary staple known as kimchi, a type of fermented cabbage.



Like many lactic acid bacteria, *L. kimchii* is capable of restricting the growth of other microorganisms by acidifying its environment, but as is common in nature, it is not content to limit itself to a single mechanism of defense. In addition to acidifying its environment, it also produces a novel antimicrobial peptide. Using modern fermentation and bioprocessing technology, **AMT** has commercialized this antimicrobial peptide to produce **Leucidal® Liquid**.

Code Number: M15008

INCI Nomenclature:

Leuconostoc/Radish Root Ferment Filtrate

INCI Status: Approved

REACH Status: Fully Compliant

CAS Number: 1686112-10-6

EINECS Number: N/A

Origin: Biotechnology/Botanical:

Leuconostoc kimchii & *Raphanus Sativus*

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,
Yellow to Light Amber Liquid

Soluble/Miscible: Water soluble

Suggested Use Levels: 2.0 - 4.0%

Suggested Applications:

Moisturization, Skin/Scalp Conditioning,
Antimicrobial



Leucidal® Liquid

US Patent Number 10,159,708

BENEFITS

A skin moisturization study was performed using an untreated control, generic cream base, and an experimental with the same cream base containing 2.0% **Leucidal® Liquid**. Comparative moisturization results from this study are shown in Figure 1. As demonstrated by the results of this study, the addition of 2.0% **Leucidal® Liquid** improved moisture levels by 42.01% after 24 hours and by 62.33% after four weeks when compared to the untreated control. When compared to the base cream **Leucidal® Liquid** improved moisturization by 14.38% and after 24 hours and by 24.13% after four weeks. Based on these results, adding this innovative product provides the formulator the opportunity to capitalize on both the natural antimicrobial properties of **Leucidal® Liquid**, as well as its ability to provide potent moisturizing benefits to the cosmetic formulation. These properties make it ideal for applications addressing numerous skin and scalp conditions.

Comparative Moisturization

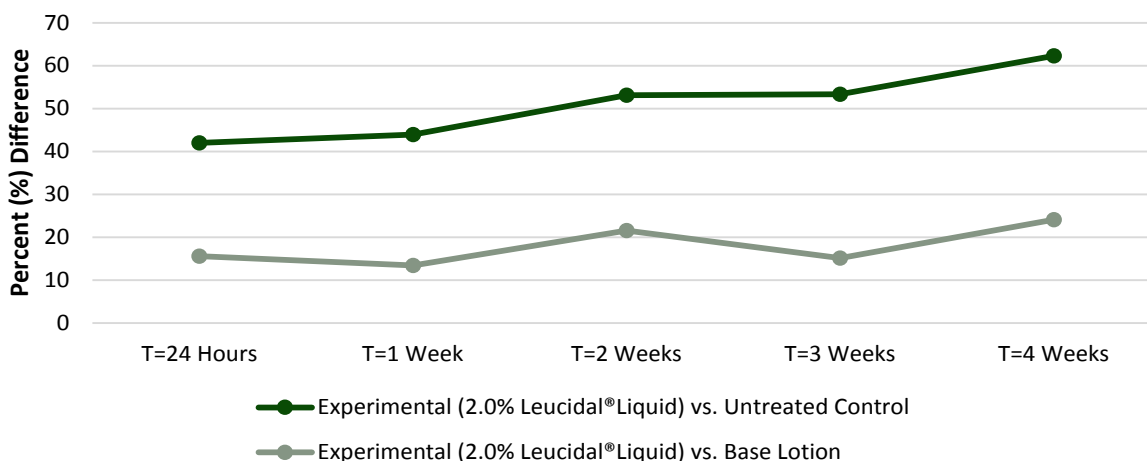


Figure 1. Percent Difference in Moisturization for **Leucidal® Liquid**

One of the first steps in the development of this product was to determine the peptide's potential ability to inhibit the growth of a variety of bacteria and fungi. Using standard serial dilution protocols in growth media, the Minimum Inhibitory Concentrations (MICs) for **Leucidal® Liquid** were determined for a variety of both bacterial and fungal organisms. The results of these tests are shown in Figure 2.

Microorganism Tested	MIC (%)
<i>E. coli</i>	2.00
<i>P. aeruginosa</i>	2.00
<i>S. aureus</i>	1.00
<i>C. albicans</i>	2.00
<i>A. brasiliensis</i>	2.00

Figure 2. MIC Data for **Leucidal® Liquid**

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% **Leucidal® Liquid** 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*. 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 microbial cultures and sampled over an additional 28-day period. Figure 3 shows the positive preservation results for 4.0% **Leucidal® Liquid** in a generic cream base formulation at pH 5.

4.0% Leucidal® Liquid in Cream Formula Challenge Test - pH 5

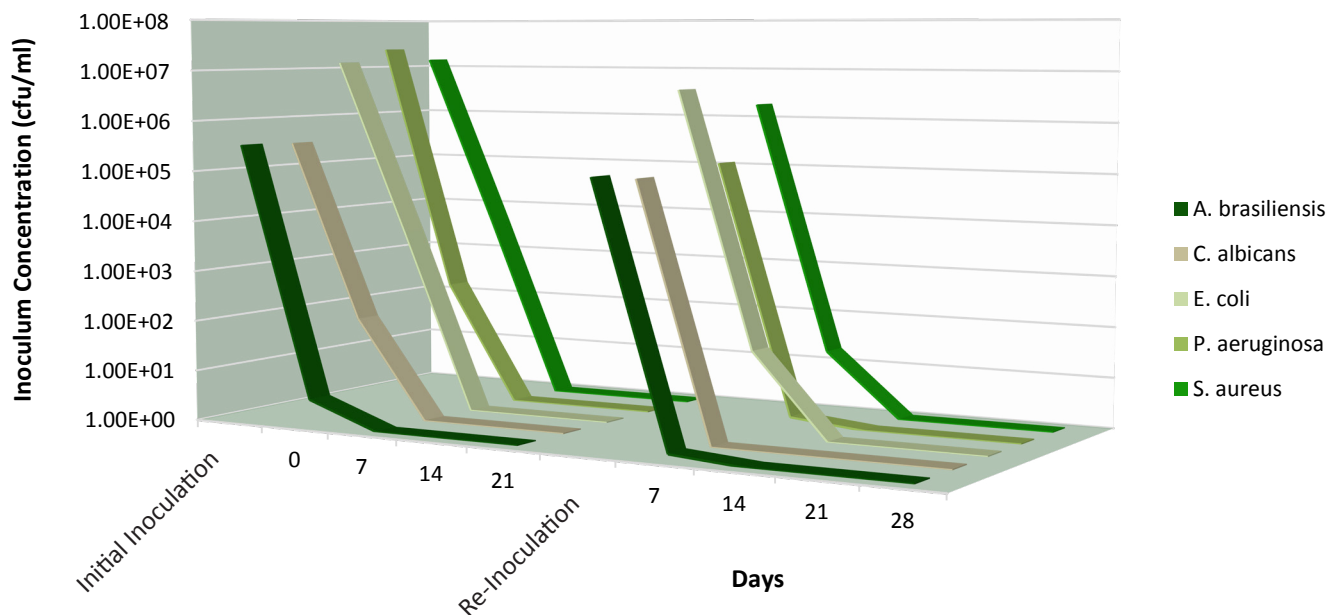


Figure 3. Challenge Test results for Generic Cream Formula pH 5 with 4.0% **Leucidal® Liquid** inoculated on Day 0 and re-inoculated on Day 28. Results show log reduction in viable organisms.

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% **Leucidal® Liquid** 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 5, 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.

4.0% Leucidal® Liquid Time Kill Test

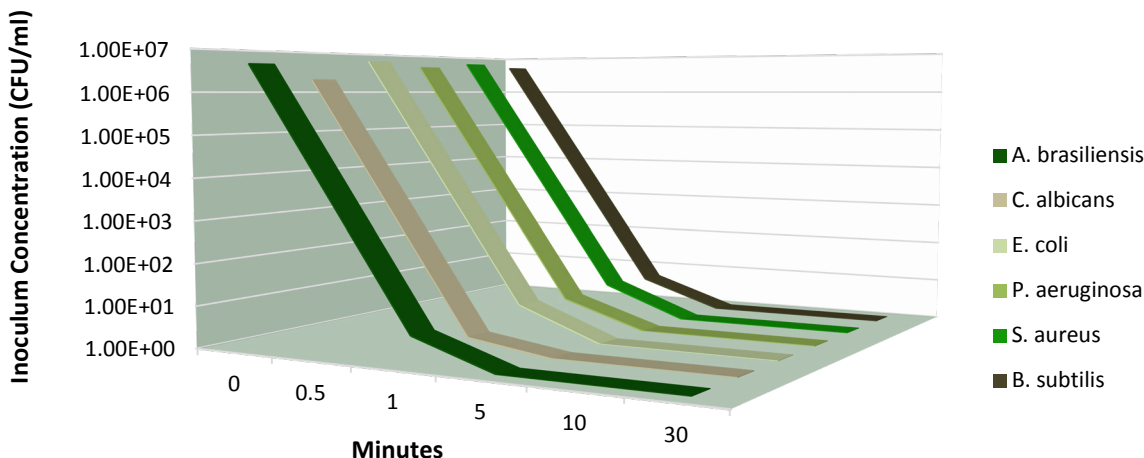


Figure 5. Time Kill Test results for 4.0% **Leucidal® Liquid**

USE RECOMMENDATIONS

As with all biological materials, some attention must be paid to the conditions under which **Leucidal® Liquid** is used. Based on bench-scale evaluations, as well as actual product applications, **Leucidal® Liquid** has been found to be effective over a wide range of typical cosmetic and personal care product manufacturing conditions. The product has been found to be heat stable up to 70°C and active under both acidic (pH 3) and basic conditions (pH 8).