ACTIVE CONCEPTS LLC
107 Technology Drive, Lincolnton | NC 28092 USA
• info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101 • https://activeconceptsllc.com/

Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product:

AC WonderShroom
21029

Test Request Form #:

12629

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in 21029 AC WonderShroom against bioburden as a function of time.

Study Dates

The study was started on February 26th, 2025 and was completed on May 2nd, 2025.

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 21029 AC WonderShroom was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

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

Organisms					
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	2.0×10^6	2.3×10^6	4.2×10^6	4.0×10^5	1.1×10^5
Day 0*	99.938%	99.999%	>99.999%	99.977%	99.781%
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	3.2×10^6	3.0×10^6	3.4×10^6	1.0×10^5	2.0×10^5
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for 21029 AC WonderShroom 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 21029 AC WonderShroom. 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 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 mold and yeast were 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 Concepts (ACL) is based on the methods published in the PCPC Microbiology Guidelines. ACL'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 ACL 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 Concepts 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 Concepts responsible for products launched based solely on the support of these studies.