# TECHNICAL SERVICE REPORT

# E5 Chem

(US)

**Report GB20220235**

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# Preservation Test SM 021

Test material:

| Samples | with |
|---|---|
| 1 | 1.0% euxyl™ pe 9010<br>0.2% sensiva™ sc 50 |
| 2 | 1.0% euxyl™ pe 9010<br>0.2% sensiva™ sc 50 |
| 3 | 1.0% euxyl™ pe 9010<br>0.2% sensiva™ sc 50 |
| 4 | 1.0% euxyl™ pe 9010<br>0.2% sensiva™ sc 50 |

Test method:

The preservation test was performed according to the enclosed standard method SM 021.

Results:

The detailed results are summarized in the following table.

Our recommendation refers to microbiological efficacy solely. A detailed safety assessment according to the EU Cosmetic Product Regulation 1223/2009 especially for the use with children under the age of three has to be performed for the individual cosmetic formulation.

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## Preservation Test SM021

| Test Material / Product | | pH | Sterility Control | Inoculation Cycles | | | | | | Assess ment |
|---|---|---|---|---|---|---|---|---|---|---|
| Index | Description | | | 1 | 2 | 3 | 4 | 5 | 6 | |
| **1** | 1.<br>1.0% euxyl™ pe 9010<br>0.2% sensiva™ sc 50 | n.a. | - | - | - | - | - | - | - | A |
| **2** | 2.<br>1.0% euxyl™ pe 9010<br>0.2% sensiva™ sc 50 | n.a. | - | - | - | - | - | - | - | A |
| **3** | 3.<br>1.0% euxyl™ pe 9010<br>0.2% sensiva™ sc 50 | n.a. | - | - | - | - | - | - | - | A |
| **4** | 4.<br>1.0% euxyl™ pe 9010<br>0.2% sensiva™ sc 50 | n.a. | - | - | - | - | - | - | - | A |

**Legend:**

| | | | | | |
|---|---|---|---|---|---|
| B | = | Bacteria | - | = | free of growth |
| M | = | Moulds | + | = | slight growth |
| Sp | = | Spore-forming bacteria | ++ | = | moderate growth |
| Y | = | Yeasts | +++ | = | heavy growth |

**Legend Assess ment:**

| | | |
|---|---|---|
| A | = | free of growth during the 6 inoculation cycles |
| B | = | slight (+) growth during the 6 inoculation cycles |
| n.a. | = | not available |

**Test start:** 15.07.2022

# Test Method SM 021 - KoKo Test

## Determination of the Preserving Effect of Chemical Preservatives in Cosmetic Formulations

The test is performed to determine the preserving effect of chemical preservatives in cosmetic formulations, e.g. in creams, lotions, sun-care products, shampoos etc.

**Principle**
With this method the efficacy of chemical preservatives is tested according to the in-can-preservation of cosmetic formulations. In separated batches different concentrations of the test preservatives are added to the unpreserved samples. A current germ load is obtained by a periodic inoculation of the test batches. Simultaneously streak cultures of each batch are made right before inoculation. The microbial growth is evaluated according to a system described below. The longer the period until microbial growth appears, the more effective the preservative.

**Performance**
Several batches are prepared in screw-topped bottles (made of LDPE) with each 25 g of the test formulation and different use concentrations of the test preservatives. (Samples which were sent for the test in a pre-preserved status are not prepared with further biocide additions.) An unpreserved sample is tested as a growth control. Two days after incorporation of the preservatives the test batches are inoculated with 0.1 ml of an inoculation solution (containing the test organisms described in the following). The titre of the solution should be $10^7$ – $10^8$ germs/ml.

| | | | | |
|---|---|---|---|---|
| **Bacteria** | Gram-positive | | *Staphylococcus aureus* | ATCC 6538 |
| | | | *Kocuria rhizophila* | ATCC 9341 |
| | Gram-negative | Enterobacteria | *Enterobacter gergoviae* | ATCC 33028 |
| | | | *Escherichia coli* | ATCC 11229 |
| | | | *Klebsiella pneumoniae* | ATCC 4352 |
| | | Pseudomonas | *Pseudomonas aeruginosa* | ATCC 9027 |
| | | | *Pseudomonas fluorescens* | ATCC 17397 |
| | | | *Pseudomonas putida* | ATCC 12633 |
| **Yeast** | | | *Candida albicans* | ATCC 10231 |
| **Moulds** | | | *Aspergillus brasiliensis* | ATCC 16404 |
| | | | *Penicillium pinophilum* | ATCC 36839 |

The test batches are both inoculated and streaked on agar plates once a week (tryptone-soya-agar for bacteria (TS) and sabouraud-dextrose-agar (SA) for yeasts and moulds). The first streak (sterility test) is done on agar plates with and without neutralizer TLSH to detect as much pre-contaminations as possible. After three days of incubation at 25 °C the microbial growth of the streak cultures is evaluated. Due to safety reasons negative streaks are observed for another two days and evaluated again. The preserving effect of the various product concentrations is judged semi-quantitatively by the growth of different streaks.

| | | | | | |
|---|---|---|---|---|---|
| - | = | free of growth | ++ | = | moderate growth |
| + | = | slight growth | +++ | = | heavy growth |

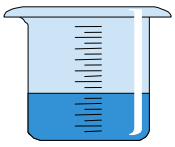
The microbial growth is classified in bacteria, yeasts and moulds. Generally the test is performed for max. six weeks, i.e. six inoculation cycles resp. stopped after manifold +++ growth.

**Evaluation of the results**
A sample can be called well preserved according criteria A, if a period of six weeks is passed under the above described laboratory conditions without showing microbial growth on the test batches. That means even after the sixth inoculation no microbial growth can be observed. From many years of experience in the use of this test method these results can state the microbiological stability of 30 months which is recommended for cosmetic products.

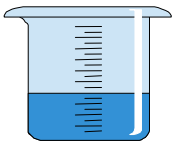
Criteria B is fulfilled if the sample showed slight microbial growth (+) during the 6 inoculation cycles. If the formulation meets criteria B, the microbiological risk analysis shall demonstrate the existence of control factors not related to the formulation; for example, a protective package such as a pump provides a higher level of protection than a jar and/or following strong demands on Good Manufacturing Practice (GMP)

# KoKo Test SM 021

25 g of each material to be tested



without preservative



with x % preservative

2 days exposure time
streak (see below) as sterility control

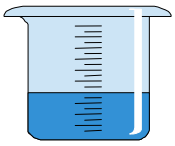
## Germ spectrum

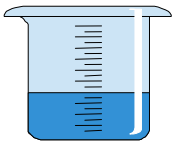
| Bacteria: | Moulds: | Yeasts: |
|---|---|---|
| **Gram-positive** | *Aspergillus brasiliensis* | *Candida albicans* |
| *Kocuria rhizophila* | *Penicillium pinophilum* | |
| *Staphylococcus aureus* | | |
| **Gram-negative** | | |
| *Enterobacter gergoviae* | | |
| *Escherichia coli* | | |
| *Klebsiella pneumoniae* | | |
| *Pseudomonas aeruginosa* | | |
| *Pseudomonas fluorescens* | | |
| *Pseudomonas putida* | | |

## Periodic microbiological preservation test

weekly inoculation with 0.1 ml mixed suspension
6 weeks = 6 inoculation cycles (titre $10^7$ – $10^8$ cfu/ml)

0,1 ml





storage at + 25 °C

Streak weekly <u>before</u> each inoculation on TS-agar and SA-agar

Incubation of the

nutrient media:

agrar plates

<u>Assessment:</u>

| | |
|---|---|
| - | free of growth |
| + | slight growth |
| ++ | moderate growth |
| +++ | heavy growth |

selective identification of:
bacteria, moulds and yeasts