Report No.: **1428/11/2022**

Date issued: 24th of November 2022

Antibacterial efficacy assessment report for the product

PROOXINE

according to PN-EN 1276:2019-12 standard

made for the company

Kemin Europa NV

Toekomstlaan 42

2200 Herental, Belgium

Ekolabos sp. z o.o. (Ltd)
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Signed by: Mateusz Latosiński Eng.
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1. INTRODUCTION

The properties of biocidal preparations, before they are authorised for use, are assessed based on tests carried out in accordance with European standards or other methods accepted by designated national authorities.

The standardisation of testing methods in recent years, through the development of successive European standards on the efficacy of disinfectants and antiseptics, allows a uniform, objective assessment of the antimicrobial effect of these agents and guarantees that the products offered in the market have adequate efficacy.

2. PURPOSE OF THE STUDY

This study aims to assess the antibacterial efficacy of the product in relation to *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442, *Escherichia coli* ATCC 10536, *Enterococcus hirae* ATCC 10541.

3. FORMAL BASIS

The assessment of antibacterial efficacy was carried out on the basis of the agreement/order dated 25th of October 2022 (Agreement No.: 44/11/2022) concluded between the Contracting Party and the Contractor.

Contracting Party:

Kemin Europa NV

Toekomstlaan 42

2200 Herental, Belgium

Contractor:

EKOLABOS sp. z o. o.

Environmental Research Laboratory

Duńska Street 9 54-427 Wrocław

Poland

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4. LEGAL BASIS

The legal basis for the conducted tests is:

The Act of 9 October 2015 on biocidal products

PN-EN 1276:2019-12 Chemical disinfectants and antiseptics **–** quantitative suspension method for the determination of the antibacterial effect of chemical disinfectants used in the food sector, industrial and domestic conditions, and in public utility facilities. Test method and requirements (phase 2, stage 1). According to the standard, the disinfectant has an antibacterial effect on the strain used if the logarithm for bacterial cell reduction obtained in the test is ≥5.

5. SAMPLE IDENTIFICATION [1]

The tested sample was the biocidal product in the form of a product for dilution. The preparation was accepted for testing on 3th of November 2022. Sample code assigned by the laboratory: 19/03/11/22. Product was delivered by the Contracting Party. After the time it was accepted for testing, and before the start of analysys the product was kept according to the storage conditions. Container was not breached until product testing started. Contractor does not bear responsibility for product stability after opening.

Product name: PROOXINE

Lot No.: no data

Product reference number: no data

Manufacturer:

Kemin Europa NV

Toekomstlaan 42

2200 Herental, Belgium

Date of manufacture: no data

Expiry date: no data

[1] Declaration by the Principal

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Product appearance: two-component product - liquid + white powder (activator) - yellow liquid after mixing

Recommended product solvent: no data

Storage conditions: no data

Active substances present in the product provided by the Contracting Party and their concentrations:

- no data

6.SCOPE OF TASKS PERFORMED

Phase 2, stage 1 assessment consists in using the dilution and neutralisation method in which the test organism is exposed to the preparation at different concentrations, time and temperatures with the addition of aggravating substances. These methods are to confirm the efficacy of the product in laboratory conditions, similar to the intended use.

6.1 CONDITIONS OF THE TEST PERFORMED

Tests performed on: 8th of November 2022– 15th of November 2022

Identification of the bacterial strains:

Staphylococcus aureus ATCC 6538,

Pseudomonas aeruginosa ATCC 15442,

Escherichia coli ATCC 10536,

Enterococcus hirae ATCC 10541.

Incubation for 24h at 37 °C ± 1 °C

Number of times the test is repeated on the microbe: 1

Required test temperature: 15°C ± 1°C

Required duration of the product contacting the bacterial suspension: 20 min± 10 sec

interfering substances: 5 mg yeast extract/L (load reduction)

Solvent used during the test: Hard Water according to EN 1276:2019-12

Stability of the product during the test:

Product stable during the study.

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6.2 TESTING METHOD AND VALIDATION

Method used: neutralisation of solutions

Counting method: deep inoculation on plates

Neutraliser used, composition: Polysorbate 80 – 30 g/l

Sodium thiosulphate – 10 g/l

Lecithin – 3 g/l

The neutralizer used allowed the method to be validated.

Substrate used: Tryptocasein Soy LAB-Agar (TSA)

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7.TESTS RESULTS

The results of product testing are presented in tables 1-2.

Table 1. Results of bacterial validation tests

Test organism	Test bacterial suspension	Validation bacterial suspension	Validation test	Control of neutraliser toxicity	Test using water
	N	**Nv_0**	**A**	**B**	**C**
Staphylococcus aureus ATCC 6538	**N: 8,61**	**Nv_0: 71**	**A: 69**	**B: 68**	**C: 67**
Pseudomonas aeruginosa ATCC 15442	**N: 8,65**	**Nv_0: 69**	**A: 67**	**B: 63**	**C: 65**
Escherichia coli ATCC 10536	**N: 8,67**	**Nv_0: 64**	**A: 62**	**B: 58**	**C: 60**
Enterococcus hirae ATCC 10541	**N: 8,68**	**Nv_0: 72**	**A: 67**	**B: 68**	**C: 69**

N – log from the number of CFU/ml put in the test suspension

Nv_0 – 1/10 of CFU/ml in the validation suspension

A – number of CFU/ml in the mixture to be validated

B – number of CFU/ml in the mixture for neutraliser toxicity test

C - number of CFU/ml in the mixture to be controlled using water and the highest concentration of the active substance

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Table 2. Test results

Test organism	N_0	Results for individual concentrations in volumetric % (test conditions: contact time: 20 minutes, temperature: 15°C ± 1°C)		
		0,4 % 2 ppm	**0,000001% 0,01 ppm**	**0,0000001% 0,001 ppm**
Staphylococcus aureus ATCC 6538	**7,61**	<14, <14	>330, >330	>330, >330
		Nx: <140 Na: <2,15	**Nx: >3300 Na: >3,52**	**Nx: >3300 Na: >3,52**
R (N_0 –Na)		**R:>5,47**	**R:<4,09**	**R:<4,09**
Pseudomonas aeruginosa ATCC 15442	**7,65**	<14, <14	>330, >330	>330, >330
		Nx: <140 Na: <2,15	**Nx: >3300 Na: >3,52**	**Nx: >3300 Na: >3,52**
R (N_0 – Na)		**R:>5,51**	**R:<4,13**	**R:<4,13**
Escherichia coli ATCC 10536	**7,67**	<14, <14	>330, >330	>330, >330
		Nx: <140 Na: <2,15	**Nx: >3300 Na: >3,52**	**Nx: >3300 Na: >3,52**
R (N_0 – Na)		**R:>5,53**	**R:<4,15**	**R:<4,15**
Enterococcus hirae ATCC 10541	**7,68**	<14, <14	>330, >330	>330, >330
		Nx: <140 Na: <2,15	**Nx: >3300 Na: >3,52**	**Nx: >3300 Na: >3,52**
R (N_0 – Na)		**R:>5,54**	**R:<4,16**	**R:<4,16**

N_0 – log (N/10); **Na** – log of CFU/ml in the test mixture after treatment
R – logarithm bacterial cell reduction obtained during the test

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Specific comments:

Verification of methodology – requirements and limits:

- N is between $1.5x10^8$ CFU/ml and $5x10^8$ CFU/ml ($8,17 \leq \log N \leq 8,70$),
- N_0 is between $1.5x10^7$ CFU/ml and $5x10^7$ CFU/ml ($7,17 \leq \log N_0 \leq 7,70$),
- N_{V0} is between 30 CFU/ml and 160 CFU/ml
- N_V is between $3.0 x10^2$ CFU/ml and $1.6 x10^3$ CFU/ml
- Control of the weighted average of the successive dilutions for N is between 5.0 and 15.0
- The average number of bacteria and yeasts, on each plate used for the calculation and obtained from the active concentration test, is between 14 and 330
- A, B and C are equal to or greater than $0.5xN_{V0}$
- At least one test concentration of the product must show a reduction log ≥5 and at least one test concentration of the product must show a reduction log <5.

8. CONCLUSIONS

The product, tested in accordance with PN-EN 1276:2019-12 standard, after contact time of 20 min, temperature of 15 °C, diluted in distilled water, with the presence of aggravating substance, shows antibacterial effect (reduction ≥5 log) in relation to:

Staphylococcus aureus	ATCC 6538	at 0,4% concentration (2 ppm)
Pseudomonas aeruginosa	ATCC 15442	at 0,4% concentration (2 ppm)
Escherichia coli	ATCC 10536	at 0,4% concentration (2 ppm)
Enterococcus hirae	ATCC 10541	at 0,4% concentration (2 ppm)

The results obtained during all controls and tests met all the requirements of the methodology and were within the limits set.

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Date issued: 24th of November 2022

Report prepared by: Mateusz Marciniak, Eng M.Sc

The results were authorised by: Jakub Jałówko, Eng, M.Sc

The report was approved by: Mateusz Latosiński, Eng

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