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CERTIFICATE OF ANALYSIS

COA No.: CT 48429/20
 COA Date: 04/03/2020
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Customer: Wildchem Chemicals (Pty) Ltd
 Order No.: n/a
 Client Reference No.: n/a
 Project No.: CT 48429/20

Analysed By: Swift Silliker Pty. Ltd. t/a Merieux NutriSciences
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Received from: Wildchem Chemicals (Pty) Ltd
 Unit 24 Silver Park, Silver Street,
 Brackenfell.

TO WILDCHEM CHEMICALS
 (PTY) LTD
 Jacques Groenewald

 jacques@wildchem.co.za

DATE RECEIVED: 19/02/2020

TEST TYPE: DISINFECTANT – BACTERICIDAL ACTIVITY (SANS 51276:2011)

METHOD NO.: SWM.MIC.015

a) Sample Identification

<input type="checkbox"/> Product Name:	GK10 BIOCID
<input type="checkbox"/> Active Ingredient:	Glutaraldehyde (10%)
<input type="checkbox"/> Batch Number:	AA2161
<input type="checkbox"/> Manufacturing Date :	n/a
<input type="checkbox"/> Exp. Date:	n/a
<input type="checkbox"/> Laboratory Number:	CT 48429/20
<input type="checkbox"/> Storage Conditions:	Ambient
<input type="checkbox"/> Appearance:	Liquid, clear, brown

Directors: V. Stewart (Managing), A. Lambrechts, P. Sans (France), S. Schneider (France), J-F. Billet (France) / Reg. No 2000/025067/07

- TMA = Total Microbial Activity / Total Viable Plate Count.
- Limit of detection of Conventional Plate Count Methods = 10CPU, unless otherwise specified.
- A test report relates only to the specific item submitted for testing. It furnishes or implies no guarantee whatsoever, in respect of a similar item that has not been tested.
- Method numbers refer to in-house methods Standard test method references available on request.
- Detection times only relevant to certain test methods where Malthus Systems are applicable.
- The test report shall not be reproduced except in full without written approval of Swift Silliker (Pty) Ltd t/a Mérieux NutriSciences.

b) Methods Used:

EN 1276:2009 – Evaluation of Bactericidal Activity
(Neutralization by dilution Method)

c) Experimental Conditions

<input type="checkbox"/> Test Strains:	<i>Escherichia coli</i> ATCC10536 <i>Enterococcus hirae</i> ATCC10541 <i>Pseudomonas Aeruginosa</i> ATCC15442 <i>Staphylococcus Aureus</i> ATCC6538
<input type="checkbox"/> Product Test Concentrations:	0.3%, 10%, 25% & Neat (Please note: a NEAT concentration is equal to 80% of sample. Products can only be tested at a concentration of 80 % or less, as some dilution is always produced by adding the test organisms and interfering substance.
<input type="checkbox"/> Appearance of Diluted Product:	0.3% - liquid, turbid, white 10% - liquid, turbid, white 25% - liquid, turbid, light brown Neat (80%) - liquid, clear, brown
<input type="checkbox"/> Interfering Substance:	3.0g/l Bovine Albumin, Dirty conditions
<input type="checkbox"/> Contact Time:	5 Minutes
<input type="checkbox"/> Test Temperature:	20°C
<input type="checkbox"/> Neutraliser:	Tween 80 (30g/l) + Lecithin (3g/l) + L-histidine (1g/l)
<input type="checkbox"/> Incubation Conditions:	Aerobic incubation: 37°C ± 1°C
<input type="checkbox"/> Incubation Media:	Tryptone Soy Agar
<input type="checkbox"/> Testing Period:	27/02-04/03/2020

d) Test Results: see tables 1-4.**e) Summary of results****Bactericidal Efficacy**

Organism	Experimental conditions	Product Conc.	Contact time	CFU/ML: Start	CFU/ML: End	Log Reduction (Log R= \geq 5)	Evaluation
<i>Enterococcus hirae</i> ATCC10541	Obligatory, Dirty conditions	0.3%	5 minutes	7.55	>3.52	<4.03	
		10%		7.55	>3.52	<4.03	
		25%		7.55	>3.52	<4.03	
		Neat (80%)		7.55	2.81	4.74	
<i>Escherichia coli</i> ATCC10536	Obligatory, Dirty conditions	0.3%	5 minutes	7.61	>3.52	<4.09	
		10%		7.61	>3.52	<4.09	
		25%		7.61	>3.52	<4.09	
		Neat (80%)		7.61	<2.15	>5.46	PASS
<i>Pseudomonas aeruginosa</i> ATCC15442	Obligatory, Dirty conditions	0.3%	5 minutes	7.39	>3.52	<3.87	
		10%		7.39	>3.52	<3.87	
		25%		7.39	>3.52	<3.87	
		Neat (80%)		7.39	<2.15	>5.24	PASS
<i>Staphylococcus Aureus</i> ATCC6538	Obligatory, Dirty conditions	0.3%	5 minutes	7.48	>3.52	<3.96	
		10%		7.48	>3.52	<3.96	
		25%		7.48	>3.52	<3.96	
		Neat (80%)		7.48	>3.52	<3.96	

f) Conclusions

According to SANS 51276, the test product, **GK10 BIOCIDE**, has no bactericidal activity ($\log R \geq 5$) under the following test conditions:

- Contact time: 5 minutes
- Temperature: 20°C
- Interfering substance: 3.0g/ L Bovine albumin – Dirty conditions
- Test strains: *Escherichia coli* ATCC 10536, *Enterococcus hirae* ATCC10541, *Pseudomonas aeruginosa* ATCC15442, *Staphylococcus Aureus* ATCC6538

Ref. Report section (d)

ORGANISM: *Enterococcus hirae* ATCC10541

Obligatory Experimental Conditions

Table 1a: Validation test

Validation suspension (N_{V0})		Experimental Conditions Control (A)= N_{VA}			Neutralizer Control (B)= N_{VB}			Method Validation (C) (Neat Product Concentration)= N_{VC}	
	Ave			Ave				Ave	
Vc1	38	Vc1	31	35.5	Vc1	38	Vc1	40	
Vc2	32	Vc2	40		Vc2	35	Vc2	44	
Acceptance limits	$N_{V0} = 30 - 160$	Acceptance limits	$\geq 0.5x N_{V0}$	Acceptance limits	$\geq 0.5x N_{V0}$	Acceptance limits	$\geq 0.5x N_{V0}$		
Complies	Yes	Complies	Yes	Complies	Yes	Complies	Yes		

$0.5 \times N_{V0} = 17.5$

Table 1b: Test suspensions

Dilution (Test suspension)	Vc1	Vc2	Average N (\bar{w}_m)	Log N	N_0	Log N_0
10^{-6}	432	300	3.6×10^8	8.55	3.6×10^7	7.55
10^{-7}	24	25				
Acceptance limits:	Log N is between 8.17 and 8.70		Complies	Yes		
Acceptance limits:	Log N_0 is between 7.17 and 7.70		Complies	Yes		
Acceptance limits:	Control of weighted mean counts: 14.1		Complies	Yes		

Table 1c: Log Reduction values

Product Concentration	Vc1	Vc2	N_a (Ave Vc1 & Vc2 x 10)	Log N_a	Log Reduction (N_0 : 7.55)	Contact time
0.3%	>330	>330	>3300	>3.52	<4.03	5 Minutes
10%	>330	>330	>3300	>3.52	<4.03	5 Minutes
25%	>330	>330	>3300	>3.52	<4.03	5 Minutes
Neat (80%)	68	60	640	2.81	4.74	5 Minutes

ORGANISM: *Escherichia coli* ATCC 10536

Table 2a: Validation test

Validation suspension (N_{v0})		Experimental Conditions Control (A)= N_{vA}		Neutralizer Control (B)= N_{vB}		Method Validation (C) (Neat Product Concentration)= N_{vC}	
	Ave		Ave		Ave		Ave
Vc1	60	Vc1	61	Vc1	60	Vc1	48
Vc2	66	Vc2	65	Vc2	80	Vc2	40
	63		63		70		44
Acceptance limits	$N_{v0} = 30 - 160$	Acceptance limits	$\geq 0.5x N_{v0}$	Acceptance limits	$\geq 0.5x N_{v0}$	Acceptance limits	$\geq 0.5x N_{v0}$
Complies	Yes	Complies	Yes	Complies	Yes	Complies	Yes
0.5 x $N_{v0} = 31.5$							

Table 2b: Test suspensions

Dilution (Test suspension)	Vc1	Vc2	Average N (wm)	Log N	N_0	Log N_0
10^{-6}	336	448	4.1×10^8	8.61	4.1×10^7	7.61
10^{-7}	52	55				
Acceptance limits:	Log N is between 8.17 and 8.70		Complies		Yes	
Acceptance limits:	Log N_0 is between 7.17 and 7.70		Complies		Yes	
Acceptance limits:	Control of weighted mean counts: 7.3		Complies		Yes	

Table 2c: Log Reduction values

Product Concentration	Vc1	Vc2	N_a (Ave Vc1 & Vc2 x 10)	Log N_a	Log Reduction ($N_0: 7.61$)	Contact time
0.3%	>330	>330	>3300	>3.52	<4.09	5 Minutes
10%	>330	>330	>3300	>3.52	<4.09	5 Minutes
25%	>330	>330	>3300	>3.52	<4.09	5 Minutes
Neat (80%)	<14	<14	<140	<2.15	>5.46	5 Minutes

ORGANISM: *Pseudomonas Aeruginosa* ATCC15442

Table 3a: Validation test

Validation suspension (N_{v0})		Experimental Conditions Control (A)= N_{vA}		Neutralizer Control (B)= N_{vB}		Method Validation (C) (Neat Product Concentration)= N_{vC}	
	Ave		Ave		Ave		Ave
Vc1	40	Vc1	30	Vc1	40	Vc1	30
Vc2	40	Vc2	28	Vc2	31	Vc2	33
	40		29		35.5		31.5
Acceptance limits	$N_{v0} = 30 - 160$	Acceptance limits	$\geq 0.5x N_{v0}$	Acceptance limits	$\geq 0.5x N_{v0}$	Acceptance limits	$\geq 0.5x N_{v0}$
Complies	Yes	Complies	Yes	Complies	Yes	Complies	Yes
0.5 x $N_{v0} = 20$							

Table 3b: Test suspensions

Dilution (Test suspension)	Vc1	Vc2	Average N (wm)	Log N	N_0	Log N_0
10^{-6}	256	224	2.5×10^8	8.39	2.5×10^7	7.39
10^{-7}	32	35				
Acceptance limits:	Log N is between 8.17 and 8.70		Complies		Yes	
Acceptance limits:	Log N_0 is between 7.17 and 7.70		Complies		Yes	
Acceptance limits:	Control of weighted mean counts: 7.2		Complies		Yes	

Table 3c: Log Reduction values

Product Concentration	Vc1	Vc2	Na (Ave Vc1 & Vc2 x 10)	Log Na	Log Reduction (No: 7.39)	Contact time
0.3%	>330	>330	>3300	>3.52	<3.87	5 Minutes
10%	>330	>330	>3300	>3.52	<3.87	5 Minutes
25%	>330	>330	>3300	>3.52	<3.87	5 Minutes
Neat (80%)	<14	<14	<140	<2.15	>5.24	5 Minutes

ORGANISM: *Staphylococcus Aureus* ATCC6538

Table 4a: Validation test

Validation suspension (Nv0)		Experimental Conditions Control (A)= NvA		Neutralizer Control (B)= NvB		Method Validation (C) (Neat Product Concentration)= NvC	
Ave		Ave		Ave		Ave	
Vc1	48	Vc1	68	Vc1	52	Vc1	40
Vc2	41	Vc2	44	Vc2	40	Vc2	40
Ave		Ave		Ave		Ave	
44.5		56		46		40	
Acceptance limits	Nv0 = 30 - 160	Acceptance limits	≥ 0.5x Nv0	Acceptance limits	≥ 0.5x Nv0	Acceptance limits	≥ 0.5x Nv0
Complies	Yes	Complies	Yes	Complies	Yes	Complies	Yes

0.5 x Nv0 = 22.25

Table 4b: Test suspensions

Dilution (Test suspension)	Vc1	Vc2	Average N (wm)	Log N	No	Log No
10 ⁻⁶	272	336	3.0 x 10 ⁸	8.48	3.0 x 10 ⁷	7.48
10 ⁻⁷	28	24				
Acceptance limits:	Log N is between 8.17 and 8.70		Complies	Yes		
Acceptance limits:	Log No is between 7.17 and 7.70		Complies	Yes		
Acceptance limits:	Control of weighted mean counts: 11.7		Complies	Yes		

Table 4c: Log Reduction values

Product Concentration	Vc1	Vc2	Na (Ave Vc1 & Vc2 x 10)	Log Na	Log Reduction (No: 7.48)	Contact time
0.3%	>330	>330	>3300	>3.52	<3.96	5 Minutes
10%	>330	>330	>3300	>3.52	<3.96	5 Minutes
25%	>330	>330	>3300	>3.52	<3.96	5 Minutes
Neat (80%)	>330	>330	>3300	>3.52	<3.96	5 Minutes

Where:

- VC = Viable Count
- N = Test suspension
- No = Test suspension at beginning of contact time (t=0)
- Na = Test suspension (survivors) before neutralization
- Nv = Validation suspension
- Nv0 = Validation suspension at beginning of contact time
- A = number of cfu/mL of the experimental conditions control
- B = number of cfu/mL of the neutralization control
- C = number of cfu/mL of the method validation

Test Validity

The test is valid when, for each test organism:


- N (Test suspension) is between $1,5 \times 10^8$ and $5,0 \times 10^8$ ($8,17 \leq \lg N \leq 8,70$)
- N_0 (Test suspension) is between $1,5 \times 10^7$ and $5,0 \times 10^7$ ($7,17 \leq \lg N_0 \leq 7,70$)
- N_{v0} is between 30 and 160 ($3,0 \times 10^1$ and $1,6 \times 10^2$)
- N_v is between $3,0 \times 10^2$ and $1,6 \times 10^3$
- A, B, C are equal to or greater than $0,5 \times N_{v0}$.
- Control of weighted mean counts: quotient is not lower than 5 and not higher than 15.
- At least one of the test concentrations will demonstrate a log reduction of less than 5 log

Pass Requirements

- For Bactericidal efficacy (as per SANS 51276), the product shall demonstrate at least a 5 decimal log reduction when diluted with hard water and tested under the other obligatory test conditions.



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