

Efficacy Bulletin
DIFFENSE™

EPA Reg. No. 5741-28

DISINFECTION DATA:

This product is bactericidal according to the AOAC Germicidal Spray Method on hard inanimate surfaces modified in the presence of 5% organic serum. Treated surfaces must remain wet for **60 seconds**

Results:

Test Organism	Sample	Sample Dilution	Number of Carriers Exposed	Number of Carriers Showing Growth
<i>Staphylococcus aureus</i> (ATCC 6538)	A >60 Days old	Ready to use	60	0
	B		60	0
	C		60	0
<i>Pseudomonas aeruginosa</i> (ATCC 15442)	A >60 Days old	Ready to use	60	1
	B		60	0
	C		60	0

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Results:

Test Organism	Sample	Sample Dilution	Number of Carriers Exposed	Number of Carriers Showing Growth
Methicillin Resistant <i>Staphylococcus aureus</i> - MRSA (ATCC 33592)	A	Ready to use	10	0
	B		10	0

This product is bactericidal according to the AOAC Germicidal Spray Method on hard inanimate surfaces modified in the presence of 5% organic serum. Treated surfaces must remain wet for **30 seconds**

Test Organism	Sample	Sample Dilution	Number of Carriers Exposed	Number of Carriers Showing Growth
<i>Acinetobacter baumannii</i> (ATCC 19606)	A	Ready to use	10	0
	B		10	0
<i>Escherichia coli</i> – with extended beta-lactamase resistance (ESBL) (ATCC BAA-196)	A	Ready to use	10	0
	B		10	0

Conclusion: In the presence of 5% organic soil load, Diffense demonstrated efficacy against the above organisms, as required by the U.S. EPA at room temperature 22.10°C.

SPOROCIDAL DATA:

This product is sporocidal according to the Quantitative Carrier Test to Determine Sporocidal Efficacy on hard inanimate surfaces modified in the presence of 5% organic serum. Treated surfaces must remain wet for **8 minutes**

Results:

Test Organism	Sample	Replicate	Log ₁₀ CFU/Carrier	Mean Log ₁₀ CFU/Carrier
<i>Clostridium difficile</i> Spores (ATCC 43598)	A	1	-0.30	0.22
		2	0.00	
		3	0.30	
		4	0.30	
		5	0.30	
		6	0.48	
		7	0.00	
		8	0.30	
		9	0.30	
		10	0.00	
	B	1	-0.30	0.20
		2	0.48	
		3	0.00	
		4	0.48	
		5	0.60	
		6	-0.30	
		7	0.30	
		8	-0.30	
		9	0.00	
		10	-0.30	
	C	1	0.00	0.08
		2	-0.30	
		3	0.00	
		4	0.00	
		5	0.30	
		6	0.00	
		7	0.30	
		8	-0.30	
		9	0.30	
		10	0.00	

Conclusion: Diffense passed the Quantitative Carrier Test when *Clostridium difficile* spores, containing the required organic load, were exposed to the test agent for eight minutes at contact temperature 22°C. All of the controls met the criteria established for a valid test. These conclusions are based on observed data.

VIRUCIDAL DATA:

This product is virucidal according to the AOAC Germicidal Spray Method on hard inanimate environmental surfaces modified in the presence of 5% organic serum. Treated surfaces must remain wet for **30 seconds**

Test Organism	Sample	Dilution	Test Substance	Input Virus Control	Dried Virus Control
Hepatitis A Virus	A	Cell Control	0000	00	0000
		10 ⁻¹	0000	++	++++
		10 ⁻²	0000	++	++++
		10 ⁻³	0000	++	++++
		10 ⁻⁴	0000	++	++++
		10 ⁻⁵	0000	++	++++
		10 ⁻⁶	0000	++	0000
		10 ⁻⁷	0000	00	0000
	TCID ₅₀ /100µL	≤10 ^{0.50}	10 ^{6.50}	10 ^{5.50}	
	B	Cell Control	0000	00	0000
		10 ⁻¹	0000	++	++++
		10 ⁻²	0000	++	++++
		10 ⁻³	0000	++	++++
		10 ⁻⁴	0000	++	++++
		10 ⁻⁵	0000	++	++++
		10 ⁻⁶	0000	++	0000
10 ⁻⁷		0000	00	0000	
TCID ₅₀ /100µL	≤10 ^{0.50}	10 ^{6.50}	10 ^{5.50}		
Human Immunodeficiency Virus Type 1 (HIV-1)	A	Cell Control	0000	00	0000
		10 ⁻¹	TTTT	++	++++
		10 ⁻²	0000	++	++++
		10 ⁻³	0000	++	++++
		10 ⁻⁴	0000	++	++++
		10 ⁻⁵	0000	++	0++0
		10 ⁻⁶	0000	++	0+00
		10 ⁻⁷	0000	00	0000
	TCID ₅₀ /200µL	≤10 ^{1.50}	10 ^{6.50}	10 ^{5.25}	
	B	Cell Control	0000	00	0000
		10 ⁻¹	TTTT	++	++++
		10 ⁻²	0000	++	++++
		10 ⁻³	0000	++	++++
		10 ⁻⁴	0000	++	++++
		10 ⁻⁵	0000	++	0++0
		10 ⁻⁶	0000	++	0+00
10 ⁻⁷		0000	00	0000	
TCID ₅₀ /200µL	≤10 ^{1.50}	10 ^{6.50}	10 ^{5.25}		

VIRUCIDAL DATA CONTINUED...



VIRUCIDAL DATA CONTINUED...

Test Organism	Sample	Dilution	Test Substance	Input Virus Control	Dried Virus Control
Influenza A (H1N1) Virus	A	Cell Control	0000	00	0000
		10 ⁻¹	0000	++	++++
		10 ⁻²	0000	++	++++
		10 ⁻³	0000	++	++++
		10 ⁻⁴	0000	++	++++
		10 ⁻⁵	0000	++	++++
		10 ⁻⁶	0000	++	000+
		10 ⁻⁷	0000	00	0000
		10 ⁻⁸	0000	00	0000
	TCID ₅₀ /100μL	≤10 ^{0.50}	10 ^{6.50}	10 ^{5.75}	
	B	Cell Control	0000	00	0000
		10 ⁻¹	0000	++	++++
		10 ⁻²	0000	++	++++
		10 ⁻³	0000	++	++++
		10 ⁻⁴	0000	++	++++
		10 ⁻⁵	0000	++	++++
		10 ⁻⁶	0000	++	000+
		10 ⁻⁷	0000	00	0000
		10 ⁻⁸	0000	00	0000
TCID ₅₀ /100μL	≤10 ^{0.50}	10 ^{6.50}	10 ^{5.75}		
Poliovirus Type 1	A	Cell Control	0000	00	0000
		10 ⁻¹	0000	++	++++
		10 ⁻²	0000	++	++++
		10 ⁻³	0000	++	++++
		10 ⁻⁴	0000	++	+++0
		10 ⁻⁵	0000	++	+000
		10 ⁻⁶	0000	++	0000
		10 ⁻⁷	NT	++	NT
		10 ⁻⁸	NT	00	NT
	TCID ₅₀ /100μL	≤10 ^{0.50}	10 ^{7.50}	10 ^{4.50}	
	B	Cell Control	0000	00	0000
		10 ⁻¹	0000	++	++++
		10 ⁻²	0000	++	++++
		10 ⁻³	0000	++	++++
		10 ⁻⁴	0000	++	+++0
		10 ⁻⁵	0000	++	+000
		10 ⁻⁶	0000	++	0000
		10 ⁻⁷	NT	++	NT
		10 ⁻⁸	NT	00	NT
TCID ₅₀ /100μL	≤10 ^{0.50}	10 ^{7.50}	10 ^{4.50}		

NT = Not Tested

VIRUCIDAL DATA CONTINUED...

Test Organism	Sample	Dilution	Test Substance	Input Virus Control	Dried Virus Control
Rotavirus	A	Cell Control	0000	00	0000
		10 ⁻¹	TTTT	++	++++
		10 ⁻²	0000	++	++++
		10 ⁻³	0000	++	++++
		10 ⁻⁴	0000	++	++++
		10 ⁻⁵	0000	++	0000
		10 ⁻⁶	0000	++	0000
		10 ⁻⁷	NT	0+	NT
		10 ⁻⁸	NT	00	NT
	TCID ₅₀ /100µL	≤10 ^{1.50}	10 ^{7.00}	10 ^{4.50}	
	B	Cell Control	0000	00	0000
		10 ⁻¹	TTTT	++	++++
		10 ⁻²	0000	++	++++
		10 ⁻³	0000	++	++++
		10 ⁻⁴	0000	++	++++
		10 ⁻⁵	0000	++	0000
		10 ⁻⁶	0000	++	0000
		10 ⁻⁷	NT	0+	NT
10 ⁻⁸		NT	00	NT	
TCID ₅₀ /100µL	≤10 ^{1.50}	10 ^{7.00}	10 ^{4.50}		

Test Organism	Dilution	Sample A		Sample B	
		Replicate #1	Replicate #2	Replicate #1	Replicate #2
Hepatitis B Virus (utilizing Duck Hepatitis B Virus as a Surrogate)	Cell Control	0000	0000	0000	0000
	10 ⁻¹	0000	0000	0000	0000
	10 ⁻²	0000	0000	0000	0000
	10 ⁻³	0000	0000	0000	0000
	10 ⁻⁴	0000	0000	0000	0000
	TCID ₅₀ /250µL	≤10 ^{0.50}	≤10 ^{0.50}	≤10 ^{0.50}	≤10 ^{0.50}
	MPN	<1.000	<1.000	<1.000	<1.000
	Log ₁₀ MPN	0.00000	0.00000	0.00000	0.00000
	MPN Log Reduction	≥5.38			
Hepatitis C Virus (utilizing Bovine Viral Diarrhea Virus (BVDV) as a surrogate)	Cell Control	0000	0000	0000	0000
	10 ⁻¹	0000	0000	0000	0000
	10 ⁻²	0000	0000	0000	0000
	10 ⁻³	0000	0000	0000	0000
	10 ⁻⁴	0000	0000	0000	0000
	TCID ₅₀ /100µL	≤10 ^{0.50}	≤10 ^{0.50}	≤10 ^{0.50}	≤10 ^{0.50}
	MPN	<1.000	<1.000	<1.000	<1.000
	Log ₁₀ MPN	0.00000	0.00000	0.00000	0.00000
	MPN Log Reduction	≥4.56			

MPN = Most Probable Number



VIRUCIDAL DATA CONTINUED...

Test Organism	Dilution	Sample A		Sample B	
		Replicate #1	Replicate #2	Replicate #1	Replicate #2
Norovirus (utilizing Feline Calicivirus as a Surrogate)	Cell Control	0000	0000	0000	0000
	10 ⁻¹	0000	0000	0000	0000
	10 ⁻²	0000	0000	0000	0000
	10 ⁻³	0000	0000	0000	0000
	10 ⁻⁴	0000	0000	0000	0000
	TCID ₅₀ /100μL	≤10 ^{0.50}	≤10 ^{0.50}	≤10 ^{0.50}	≤10 ^{0.50}
	MPN	<1.000	<1.000	<1.000	<1.000
	Log ₁₀ MPN	0.00000	0.00000	0.00000	0.00000
	MPN Log Reduction	≥5.93			

MPN = Most Probable Number

Conclusion: In the presence of 5% organic soil load, Diffense demonstrated complete inactivation of the above viruses at room temperature (21.0°C), as required by the U.S. EPA for virucidal label claims.