

Vaccinia virus (VR-1549) Elstree strain Test Results

| EN14476:2013 + A2:2019 Suspension test for the efficacy of Safe4 Disinfectant Cleaner, Batch 4661, BT-SAF-21 from Safe Solutions (Safe4) Ltd. against Vaccinia virus VR-1549 under clean conditions | | | | | | |
|---|--------|------------------------|--------|------------------------|--------|------------------------|
| Test Results | | | | | | |
| Concentration | 1:500 | | 1:50 | | 1:10 | |
| Exposure Time | data | TCID ₅₀ /ml | data | TCID ₅₀ /ml | data | TCID ₅₀ /ml |
| t = 5 mins | 2.33 | 6.81E+03 | 0.00 | 3.16E+01 | 0.00 | 3.16E+01 |
| Raw Data | 662000 | 6.81E+03 | 000000 | 3.16E+01 | 000000 | 3.16E+01 |
| log | | 3.83 | | 1.50 | | 1.50 |
| log difference | | 1.67 | | 4.00 | | 4.00 |

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|---|-----------------------|---------------|-----------------------|-----------------------|-------|--------|--------|--------|-------------------------------|
| Summary Table | | | | | | | | | |
| Product: | Interfering substance | Concentration | Level of cytotoxicity | lg TCID ₅₀ | | | | | >4 lg reduction after 'X' Min |
| | | | | 0 min | 5 min | 15 min | 30 min | 60 min | |
| Safe4 Disinfectant Cleaner | 0.3g/l BSA | 1:10 | 1.50 | 2.33 | 1.50 | n.a. | n.a. | n.a. | 5 mins |
| | | 1:50 | 1.50 | n.a. | 1.50 | n.a. | n.a. | n.a. | 5 mins |
| | | 1:500 | 1.50 | n.a. | 3.83 | n.a. | n.a. | n.a. | >5 mins |
| Virus Control | CLEAN | | | 5.67 | 5.50 | 5.67 | n.a. | n.a. | n.a. |
| | | | | | | | 5 min | 15 min | |
| Formaldehyde | PBS | 0.7% (w/v) | 3.50 | | | | 4.50 | 3.50 | >15 mins |

Vaccinia virus (VR-1549) Elstree strain Control Data

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|---|------------------------|----------------------|------------------------|------------------------|------------------------|------------------------|------------------------|-----------------------------------|------------------------|------------------------------|------------------------|------------------------|
| Controls | | | | | | | | | | | | |
| Virus Recovery 0 min | | Virus Recovery 5 min | | Virus Recovery 15 min | | Cytotoxicity | | Disinfectant Suppression VS | | Disinfectant Suppression VS2 | | |
| raw data | TCID ₅₀ /ml | raw data | TCID ₅₀ /ml | raw data | TCID ₅₀ /ml | raw data | TCID ₅₀ /ml | raw data | TCID ₅₀ /ml | raw data | TCID ₅₀ /ml | |
| 4.17 | 4.64E+05 | 4.00 | 3.16E+05 | 4.17 | 4.64E+05 | 0.00 | 3.16E+01 | 0.83 | 2.15E+02 | 4.33 | 6.81E+05 | |
| 666610 | 4.64E+05 | 666600 | 3.16E+05 | 666610 | 4.64E+05 | 000000 | 3.16E+01 | 320000 | 2.15E+02 | 666620 | 6.81E+05 | |
| | 5.67 | | 5.50 | | 5.67 | | 1.50 | | 2.33 | | 5.83 | |
| | | | | | | | | | 3.17 | | -0.33 | |
| Formaldehyde reference inactivation controls | | | | | | | | | | | | |
| Cytotoxicity | | Exposure time | 0.7% Formaldehyde | | | | No column Control | | | | | |
| | | | 5 mins | | 15 mins | | 5 mins | | | | | |
| raw data | TCID ₅₀ /ml | | raw data | TCID ₅₀ /ml | raw data | TCID ₅₀ /ml | raw data | TCID ₅₀ /ml | raw data | TCID ₅₀ /ml | raw data | TCID ₅₀ /ml |
| 2.00 | 3.16E+03 | | 3.00 | 3.16E+04 | 2.00 | 3.16E+03 | | | 4.33 | 6.81E+05 | | |
| 660000 | 3.16E+03 | | 666000 | 3.16E+04 | 660000 | 3.16E+03 | | | 666620 | 6.81E+05 | | |
| | 3.50 | log | | 4.50 | | 3.50 | | | | 5.83 | | |
| | | log difference | | 1.17 | | 2.17 | | | | | | |
| Interference control | | Virus dilution | | | | | | Stock Virus (TCID ₅₀) | | | | |
| | | -3 | -4 | -5 | -6 | -7 | -8 | 5.50 | | | | |
| PBS Control | | 1 | 1 | 1 | 0.67 | 0 | 0 | 1.00E+07 | | | | |
| | | 3.16E+02 | 3.16E+02 | 3.16E+02 | 1.48E+02 | 3.16E+01 | 3.16E+01 | 66666210 | | | | |
| Raw Data | | 2.50 | 2.50 | 2.50 | 2.17 | 1.50 | 1.50 | | | | | |
| | | 6 | 6 | 6 | 4 | 0 | 0 | | | | | |
| Product | | 1 | 1 | 1 | 0.83 | 0 | 0 | | | | | |
| | | 3.16E+02 | 3.16E+02 | 3.16E+02 | 2.14E+02 | 3.16E+01 | 3.16E+01 | | | | | |
| Raw Data | | 2.50 | 2.50 | 2.50 | 2.33 | 1.50 | 1.50 | | | | | |
| | | 6 | 6 | 6 | 5 | 0 | 0 | | | | | |
| Log Difference | | 0.00 | 0.00 | 0.00 | -0.16 | 0.00 | 0.00 | | | | | |
| Product Cyt Dilution | | -1 | -1 | -1 | -1 | -1 | -1 | | | | | |
| PBS Dilution | | Neat | Neat | Neat | Neat | Neat | Neat | | | | | |

CONCLUSION

Verification of the methodology

A test is only valid if the following criteria are fulfilled:

- a) The titre of the test suspension of at least 10^8 TCID₅₀ /ml is sufficiently high to at least enable a titre reduction of 4 lg to verify the method.
- b) Detectable titre reduction is at least 4 log₁₀.
- c) Difference of the logarithmic titre of the virus control minus the logarithmic titre of the test virus in the reference inactivation test is between:
 - Between 0.75 and 3.5 after 5 min and between 2.0 and 4.0 after 15 min for Vaccinia virus
- d) Cytotoxicity of the product solution does not affect cell morphology and growth or susceptibility for the test virus in the dilutions of the test mixtures which are necessary to demonstrate a 4 log₁₀ reduction of the virus.
- e) The interference control result does not show a difference of > 1.0 log₁₀ of virus titre for test product treated cells in comparison to the non-treated cells.
- f) Neutralisation validation. This is called the disinfectant suppression test in this protocol. The disinfectant was neutralised by column chromatography through an Illustra Microspin S-400 HR column to achieve the best possible neutralisation available for this test. The difference for virus is greater than 0.5 log₁₀ indicating rapid irreversible virucidal activity of the disinfectant by dilution at a concentration of 1:10 for VS1. This neutralisation validation has been verified by VS2, which shows the product has been successfully neutralised.

According to EN 14476:2013 + A2:2019, **Safe4 Disinfectant Cleaner POSSESSES VIRUCIDAL** activity at concentrations of **1:50 and 1:10** as tested after **5 MINUTES** at **20°C** under **CLEAN** conditions (0.3 g/l bovine albumin) against *Vaccinia virus* VR-1549 Elstree strain / Vero cells.

This product therefore is effective against all enveloped viruses as defined in EN 14476:2013 + A2:2019 Annex A*. This therefore includes all coronaviruses and SARS-CoV-2.

Authorised signatory



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DISCLAIMER

The results in this test report only pertain to the sample supplied.

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***EN 14476 2013 + A2 2019 Annex A (informative – Enveloped viruses)**

Poxviridae
Herpesviridae
Filoviridae (e.g. Ebola, Marburg)
Flavivirus
Hepatitis C Virus (HCV)
Hepatitis Delta Virus (HDV)
Influenza Virus
Paramyxoviridae
Rubella Virus
Measles Virus
Rabies Virus
Coronavirus (e.g. SARS, MERS)
Human Immunodeficiency Virus (HIV)
Human T Cell Leukemia Virus (HTLV)
Hepatitis B virus (HBV)

Reference: Van Regenmortel MHV et al.,Eds.: Virus Taxonomy, Classification and Nomenclature of Viruses, seventh report of the international committee on taxonomy of viruses. Academic Press, San Diego, 2000