**MOISTURE 22 CO., LTD**
No. 10 Lane 231 Nankan
Rd. Sec. 1 Luchu district
TAOYUAN CITY 338
TAIWAN

# DETERMINATION OF THE VIRUCIDAL ACTIVITY OF THE FLE-05 PRODUCT ACCORDING TO THE EN 14476+A2:07-2019 STANDARD

# ANALYSIS REPORT
## R/20/20400

| Version # | Validation - Scientific & Technical Direction / Quality Direction | Verification – Assistant of the Scientific & Technical Direction | Edition date of the version | Date of amendment |
|---|---|---|---|---|
| 1 | J.-F. LACROIX | J.-F. LACROIX | 21/10/2020 | |




43 Route du Polygone 67100 STRASBOURG – France – Tél : 03 90 41 29 80 – Fax : 03 90 41 29 83 – E-mail : contact@analytice.com
SARL au capital de 40 000 Euros – RCS Strasbourg TI B 439 223 256 - SIRET 439 223 256 00033 – APE 7120 B – TVA intra FR 52 439 223 256

## TABLE OF CONTENTS



## APPENDIX



## SAMPLES IDENTIFICATION

| Our references | Information provided by the client | | Date of manufacturing | Date of analysis |
|---|---|---|---|---|
| | Your references | Description | | |
| E/20/81632 | FLE-05 (FLE-XXX (X=1-999) the number means amount of liquid | Disinfectant solution | 08/2020 | From 30/08/2020 to 02/10/2020 |

**Your order:** Quotation 201918-1 signed

## 1. Mission

The firm MOISTURE 22 CO., LTD asks the firm Analytice to undertake a study to test the virucidal activity of the product **FLE-05** according to the EN 14476+A2:07-2019 standard: chemical antiseptics and disinfectants – virucidal quantitative suspension tests for chemical disinfectants and antiseptics used in human medicine.

## 2. Sample identification

- Manufacturer: MOISTURE 22 CO., LTD.
- Manufacturing date: 08/2020.
- Expiration date: 08/2025.
- Storage condition: room temperature.
- Active substances: 2-phenoxyethanol 0,5% (CAS n°122-99-6).
- Appearance of the product: liquid, colorless.
- Product diluent recommended by the manufacturer for use: none, ready-to-use product.
- Date of delivery of the product: 27/08/2020.
- Date of test: from 30/08/2020 to 02/10/2020.

## 3. Experimental conditions

- Standard: EN 14476+A2:07-2019.
- Temperature used during the assays: 20°C ± 1°C.
- Titration units: log $TCID_{50}$.
- Exposure time: 30 s (clean conditions) - 30 min (dirty conditions).
- Final concentrations tested: 80% - 10%.
- Diluent used for the product: distilled water.
- Viral strains: **Vaccinia virus**, grown on BHK-21 cells, under 5% $CO_2$ atmosphere.
- Organic soil load: 0,3 g/L bovine serum albumin (80% and 10 %, 30 s, clean conditions) and
  3 g/L bovine serum albumin + 3 mL/L sheep erythrocytes (80% and 10 %, 30 min, dirty conditions).
- Product stability: good.
- Stop solution: cold shock.

**Viral titre:**

**Viral titers are expressed in log $TCID_{50}$** (calculated by Spearman-Kärber method)**:** 6,500 log $TCID_{50}$

# 4. Method validation

## 4.1. Cytotoxicity

For Vaccinia virus, the BHK-21 cells were exposed to the FLE-05 product and a weak cytotoxic effect was observed at the dilution $10^{-1}$.

## 4.2. Susceptibility to viruses

The viruses were titrated on cell cultures untreated with the FLE-05 product (indicator cell line) and titrated on cell cultures treated with the FLE-05 product.

| **VACCINIA VIRUS** | | **Viral titer (log $TCID_{50}$)** | | |
|---|---|---|---|---|
| | | Untreated cell cultures | Treated cell cultures | **Viral titer (log $TCID_{50}$)** |
| **FLE-05** | $10^{-2}$ | 6,500 | 6,000 | **0,500** |

According to the European standard EN 14476+A2:07-2019, the FLE-05 product used at the dilution of $10^{-2}$ does not have an effect on the Vaccinia virus titration method (the difference between viral titers must be < 1,0 log).

## 4.3. Validations of the cold shock method

The method is validated if the difference between viral titres is ≤ 0,5 log.

| Product concentration | **Organic soil load** | **Viral titer (log $TCID_{50}$)** | **Difference with the viral suspension** |
|---|---|---|---|
| **FLE-05 80%** | 0,3 g/L BSA | TRIAL 1: 6,500 | 0,000 |
| | | TRIAL 2: 6,500 | 0,000 |

| Product concentration | **Organic soil load** | **Viral titer (log $TCID_{50}$)** | **Difference with the viral suspension** |
|---|---|---|---|
| **FLE-05 80%** | 3 g/L BSA + 3 mL/L sheep erythrocytes | TRIAL 1: 6,500 | 0,000 |
| | | TRIAL 2: 6,500 | 0,000 |

## 4.4. Inactivation assays of the virus with a control solution

| **Formaldehyde 0,7%** | **Viral titer (log $TCID_{50}$)** | **Viral titer reduction (log $TCID_{50}$)** |
|---|---|---|
| **Viral suspension control** | 6,500 | |
| Inactivation 5 min | 5,875 | 0,625 |
| Inactivation 15 min | 5,375 | 1,125 |
| Inactivation 30 min | 4,875 | **1,625** |

The viral titer reduction (difference between the titers of the viral suspension treated with 0,7 % formaldehyde and the viral suspension control) must be between -0,5 and -2,5 log after 30 min of exposure. In the assays, the viral titer reduction is 1,625 log, indicated that the Vaccinia virus is inactivated after 30 min of exposure with the 0,7 % formaldehyde solution.

## 5. Testing - Calculation of the virucidal activity

### *Trial 1*

The viral suspension was titrated at **6,500 log $TCID_{50}$.**

| PRODUCT | Concentration | Time of exposure | Organic soil load | Temperature | **Viral titer (log $TCID_{50}$)** | **Viral titer reduction** |
|---|---|---|---|---|---|---|
| **FLE-05** | 80% | 30 s | 0,3 g/L BSA | 20°C | 2,375 | **4,125** |
| | 10% | | | | 5,625 | 0,875 |
| | 80% | 30 min | 3 g/L BSA + 3 mL/L sheep erythrocytes | | 1,750 | **4,750** |
| | 10% | | | | 5,000 | 1,500 |

*The tested concentrations of product have a virucidal effect if the reduction in the viral titer is greater than or equal to 4.0 log.*

### *Trial 2*

The viral suspension was titrated at **6,500 log $TCID_{50}$.**

| PRODUCT | Concentration | Time of exposure | Organic soil load | Temperature | **Viral titer (log $TCID_{50}$)** | **Viral titer reduction** |
|---|---|---|---|---|---|---|
| **FLE-05** | 80% | 30 s | 0,3 g/L BSA | 20°C | 2,375 | **4,125** |
| | 10% | | | | 5,750 | 0,750 |
| | 80% | 30 min | 3 g/L BSA + 3 mL/L sheep erythrocytes | | 1,625 | **4,875** |
| | 10% | | | | 5,375 | 1,125 |

*The tested concentrations of product have a virucidal effect if the reduction in the viral titer is greater than or equal to 4.0 log.*

## 6. Methodology validation

The assays were validated as required by the European standard EN 14476+A2:07-2019:

- The viral titers of the suspension tests were sufficient in order to observe a reduction of 4 log after time exposure with the product:
  - 6,500 log $TCID_{50}$ for Vaccinia virus.

- The virus was inactivated with the control solution of 0,7 % formaldehyde after 30 min of exposure:
  - the reduction observed was of 1,625 log for the Vaccinia virus.

- The FLE-05 product does not have a cytotoxic effect on the BHK-21 cells.

- The tested product does not affect the infectious capacity of the viruses:
  - For Vaccinia virus, the differences in viral titers between the virus inoculated on BHK-21 cells and the virus inoculated on the BHK-21 cells treated with the product was ≤ 1,0 log (0,500 log).

## 7. Conclusion

The assays performed with the **FLE-05** product:

**1.** Demonstrated a **virucidal activity** on the **Vaccinia virus** from the concentration **80%**, as required by the European standard EN 14476+A2:07-2019, following a **30 s** exposure period, at **20°C**, in **clean** conditions.

- That in accordance with the standard EN 14476+A2:07-2019, the product **FLE-05** is therefore **effective on all enveloped viruses (including H1N1 and coronavirus)** from **80%**, following a **30 s** exposure period, at **20°C**, in **clean** conditions (point 4, Table 1 page 9 “The trial on '' enveloped viruses virucidal activity [with Vaccinia virus model viruses]'' will cover all enveloped viruses”)

**2.** Demonstrated a **virucidal activity** on the **Vaccinia virus** from the concentration **80%**, as required by the European standard EN 14476+A2:07-2019, following a **30 min** exposure period, at **20°C**, in **dirty** conditions.

- That in accordance with the standard EN 14476+A2:07-2019, the product **FLE-05** is therefore effective on **all enveloped viruses (including H1N1 and coronavirus)** from **80%**, following a **30 min** exposure period, at **20°C**, in **dirty** conditions (point 4, Table 1 page 9 “The trial on '' enveloped viruses virucidal activity [with Vaccinia virus model viruses]'' will cover all enveloped viruses”

---

APPENDIX

**Moisture 22 Co., Ltd.**
10, Lane 231,Sec.1, Nankan Rd., Luchu, Taoyuan, Taiwan.
Tel : +886 3 2227470 / FAX : +886 3 2227469

# APPENDIX 1

**Vaccinia virus:**

- Cell line: BHK 21 cells (HPA ref. 85011433, batch n°09I007)
- Viral strain: Vaccinia virus (ATCC ref. VR-1508, batch n°5016818)

**Buffers and media:**

- PBS buffer: sodium chloride, Panreac, ref. 141659.1211, batch n° 0000204679; sodium phosphate dibasic, Sigma Aldrich, ref. S5136, batch n° BCBC7067V; sodium phosphate monobasic, Sigma Aldrich, ref. S5011, batch n° 1019K01021V

- MEM media, Sigma Aldrich, ref. 0268, batch n° 040M8301

- DMEM media, Sigma Aldrich, ref. D5796, batch n° RNBB9336

- Fetal calf sera, Sigma Aldrich, F7524, batch n° 098K3397

**Reagents:**

- Albumine bovine sera, Sigma Aldrich, ref. 05479, batch n° STBB7838V

**Inactivation solution:**

- Formaldehyde, Sigma Aldrich, ref. F-1635, batch n° BCBB3510

## APPENDIX 2

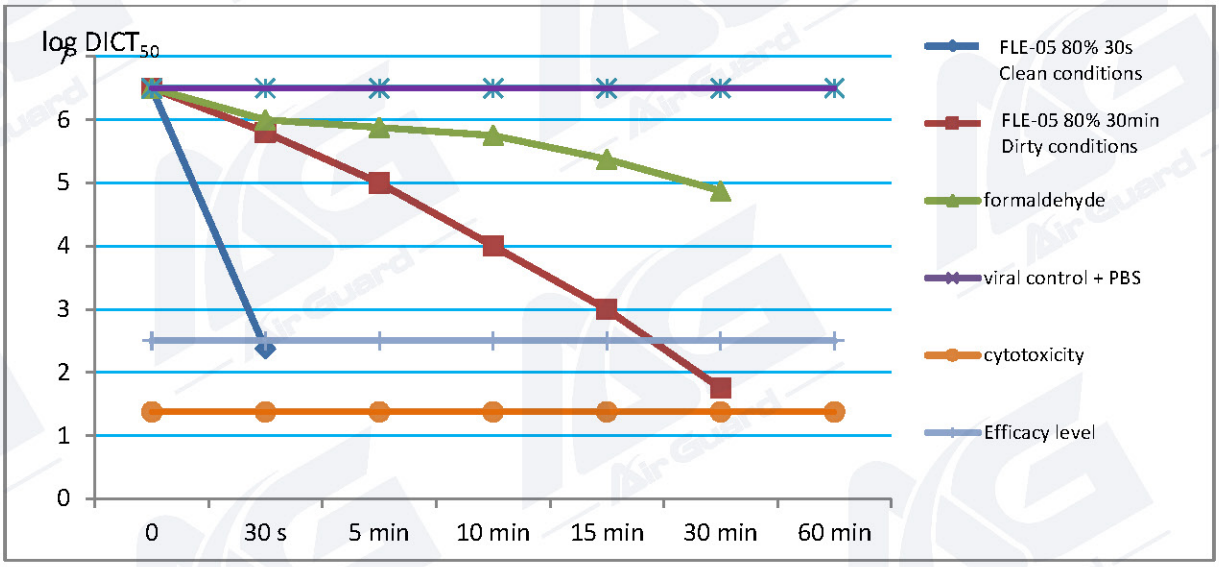
**Table A1** – **Vaccinia virus** titer, by Spaerman-Kärber method:

Log $TCID_{50}$ = 6,500

| Dilution (- log) | Result | % positive results |
|---|---|---|
| -3 | 44444444 | 100 |
| -4 | 44444444 | 100 |
| -5 | 44444444 | 100 |
| -6 | 44444444 | 100 |
| -7 | 00000000 | 0 |
| -8 | 00000000 | 0 |
| -9 | 00000000 | 0 |
| -10 | 00000000 | 0 |
| Sum of % positive results | | 400 |

**Chart 1 –** Trials on **Vaccinia virus**, graphic representation of the results obtained

*Trial 1*




*Trial 2*

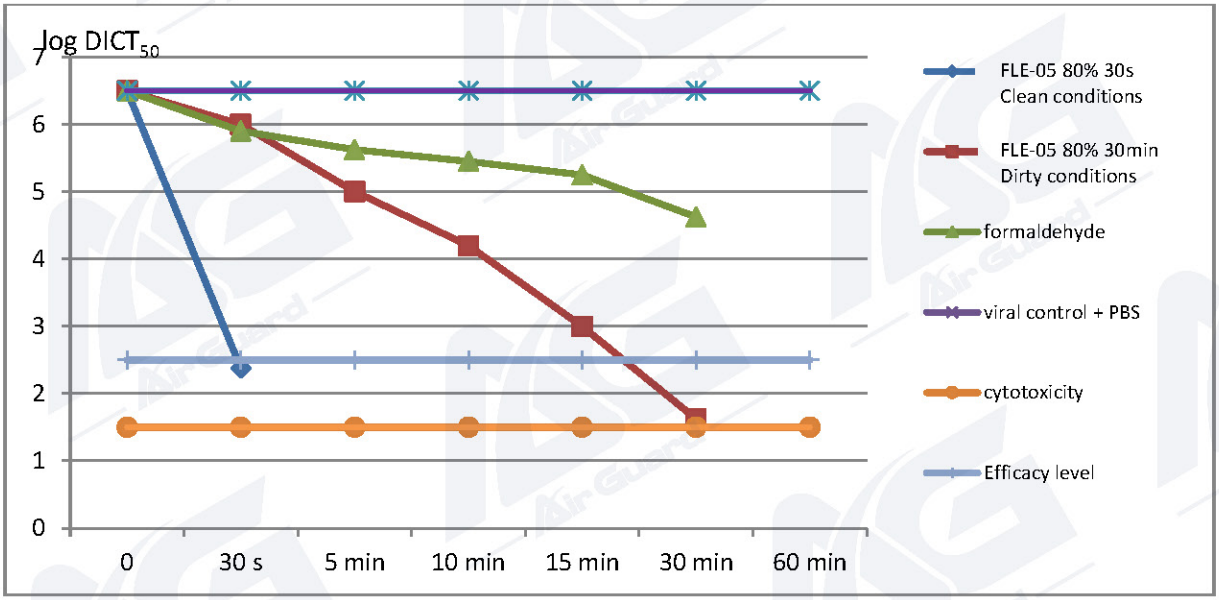


**Table A2 -** Results on ***Vaccinia virus*** in clean conditions (0,3 g/l BSA)

| PRODUCT | Concentration | Organic soil load | Cytotoxicity level | Lg $TCID_{50}$ | | | | | | | Reduction |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 | 30 s | 5 min | 10 min | 15 min | 30 min | 60 min | |
| **FLE-05** TRIAL 1 | 80,00% | 0,3 g/L BSA | 1,375 | 6,500 | 2,375 | N.T. | N.T. | N.T. | N.T. | N.T. | 30 s R = 4,125 |
| **FLE-05** TRIAL 2 | 80,00% | 0,3 g/L BSA | 1,500 | 6,500 | 2,375 | N.T. | N.T. | N.T. | N.T. | N.T. | 30 s R = 4,125 |
| **FLE-05** TRIAL 1 | 80,00% | 3 g/L BSA + 3 mL/L sheep erythrocytes | 1,375 | 6,500 | N.T. | N.T. | N.T. | N.T. | 1,750 | N.T. | 30 min R = 4,750 |
| **FLE-05** TRIAL 2 | 80,00% | 3 g/L BSA + 3 mL/L sheep erythrocytes | 1,500 | 6,500 | N.T. | N.T. | N.T. | N.T. | 1,625 | N.T. | 30 min R = 4,875 |
| Formaldehyde TRIAL 1 | 0,70% | PBS | 1,625 | 6,500 | N.T. | 5,875 | N.T. | 5,375 | 4,875 | N.T. | |
| Formaldehyde TRIAL 2 | 0,70% | PBS | 1,750 | 6,500 | N.T. | 5,625 | N.T. | 5,250 | 4,625 | N.T. | |
| VIRAL CONTROL OF INFECTIVITY TRIAL 1 | N.A. | 0,3 g/L BSA | N.A. | 6,500 | N.T. | N.T. | N.T. | N.T. | N.T. | 6,500 | |
| VIRAL CONTROL OF INFECTIVITY TRIAL 1 | N.A. | 3 g/L BSA + 3 mL/L sheep erythrocytes | N.A | 6,500 | N.T. | N.T. | N.T. | N.T. | N.T. | 6,500 | |
| VIRAL CONTROL OF INFECTIVITY TRIAL 2 | N.A. | 0,3 g/L BSA | N.A | 6,500 | N.T. | N.T. | N.T. | N.T. | N.T. | 6,500 | |
| VIRAL CONTROL OF INFECTIVITY TRIAL 2 | N.A. | 3 g/L BSA + 3 mL/L sheep erythrocytes | N.A | 6,500 | N.T. | N.T. | N.T. | N.T. | N.T. | 6,500 | |
| CELL SENSITIVITY TO THE VIRUS | $10^{-2}$ | N.A. | Untreated cells | 6,500 | | | | | | | |
| | | N.A. | Treated cells | 6,000 | | | | | | | |

**Table A3** - Raw results for trials 1 and 2

*TRIAL 1*

| | Concentration | Organic soil load | Exposure time | Dilutions | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | -1 | -2 | -3 | -4 | -5 | -6 | -7 | -8 | -9 |
| **FLE-05** TRIAL 1 | 80,00% | 0,3 g/L BSA | 30 s | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4440 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
| | 10,00% | | | 4444 | 4444 | 4444 | 4444 | 4444 | 4000 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 |
| | 80,00% | 3 g/L BSA + 3 mL/L sheep erythrocytes | 30 min | 4444 | 4400 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
| | | | | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
| | 10%% | | | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 |
| | VIRAL CONTROL | | | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| **FLE-05** cytotoxicity | 80,00% | 0,3 g/l BSA | N.A. | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
| | | | | 4440 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
| Formaldehyde | 0,70% | PBS | 5 | 4444 | 4444 | 4444 | 4444 | 4444 | 4440 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 |
| | | | 15 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4440 | 0000 | 0000 | 0000 | 0000 |
| | | | 30 | 4444 | 4444 | 4444 | 4444 | 4440 | 0000 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 |
| Formaldehyde (cytotoxicity) | 0,70% | PBS | N.A. | 4444 | 4000 | 0000 | 0000 | N.T. | N.T. | N.T. | N.T. | N.T. |
| | | | | 4444 | 0000 | 0000 | 0000 | | | | | |
| VIRAL CONTROL OF INFECTIVITY | N.A. | 0,3 g/l BSA | 0 | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | 60 | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| VIRAL CONTROL OF INFECTIVITY | N.A. | 3 g/L BSA + 3 mL/L sheep erythrocytes | 0 | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | 60 | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |

*TRIAL 2*

| | Concentration | Organic soil load | Exposure time | Dilutions | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | -1 | -2 | -3 | -4 | -5 | -6 | -7 | -8 | -9 |
| **FLE-05** TRIAL 2 | 80,00% | 0,3 g/L BSA | 30 s | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4440 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
| | 10,00% | | | 4444 | 4444 | 4444 | 4444 | 4444 | 4400 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 |
| | 80,00% | 3 g/L BSA + 3 mL/L sheep erythrocytes | 30 min | 4444 | 4000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
| | | | | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
| | 10,00% | | | 4445 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4440 | 0000 | 0000 | 0000 | 0000 |
| | VIRAL CONTROL | | | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| **FLE-05** cytotoxicity | 80,00% | 0,3 g/l BSA | N.A. | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
| | | | | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
| Formaldehyde | 0,70% | PBS | 5 | 4444 | 4444 | 4444 | 4444 | 4444 | 4000 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 |
| | | | 15 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4400 | 0000 | 0000 | 0000 | 0000 |
| | | | 30 | 4444 | 4444 | 4444 | 4444 | 4000 | 0000 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 |
| Formaldehyde (cytotoxicity) | 0,70% | PBS | N.A. | 4444 | 4400 | 0000 | 0000 | N.T. | N.T. | N.T. | N.T. | N.T. |
| | | | | 4444 | 0000 | 0000 | 0000 | | | | | |
| VIRAL CONTROL OF INFECTIVITY | N.A. | 0,3 g/L BSA | 0 | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | 60 | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| VIRAL CONTROL OF INFECTIVITY | N.A. | 3 g/L BSA + 3 mL/L sheep erythrocytes | 0 | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | 60 | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |

*Sensitivity of the cells to the Vaccinia virus:*

| PRODUCT | Dilution | ORGANIC SOIL LOAD | | Dilutions | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | -2 | -3 | -4 | -5 | -6 | -7 | -8 | -9 |
| **FLE-05** | $10^{-2}$ | 0,3 g/l BSA | Untreated cells | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | Treatde cells | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 |

| PRODUCT | Dilution | ORGANIC SOIL LOAD | | Dilutions | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | -2 | -3 | -4 | -5 | -6 | -7 | -8 | -9 |
| **FLE-05** | $10^{-2}$ | 3 g/L BSA + 3 mL/L sheep erythrocytes | Untreated cells | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | Treatde cells | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
| | | | | 4444 | 4444 | 4444 | 4444 | 4400 | 0000 | 0000 | 0000 |