



SUBJECT In Vitro Cytotoxicity Test

TEST LOCATION TÜV SÜD China

TÜV SÜD Products Testing (Shanghai) Co., Ltd.
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CLIENT NAME 常州市彭义纺织品有限公司

CLIENT ADDRESS 江苏省常州市武进区科教城大连理工 A701

TEST PERIOD 14-Dec-2020~08-Jan-2021

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RECEIPT DATE / TEST DATE

14-Dec-2020/ 14-Dec-2020

THE FOLLOWING SAMPLE(S) WAS/WERE SUBMITTED

BY/ ON BEHALF OF THE CLIENTS AS:

Sample Name: oxford fabric
Sample Specification: 黑色 600D
Batch No./Date: /
Manufacturer: 常州市彭义纺织有限公司

SAMPLE NO.	DESCRIPTION	PHOTOGRAPH
721660480	PU	

SUMMARY

1. Purpose

The purpose of this test is to determine the biological reactivity of a mammalian cell culture (mouse fibroblast L-929 cells) in response to the test article Oxford fabric extract.

2. Process Description

The suspended cells were dispensed in 96-well plate, and incubated in cell incubator (5% CO₂, 37°C) on the first day.

On the second day, the test article extract (100%, 75%, 50% and 25% in growth medium) was added to L-929 cells in 96-well plates and then incubated at 37°C in 5% CO₂ for another 24h.

After 24h incubation, observed the cell morphology first and 50 µL aliquot of MTT (1 mg/mL) was added. 2h incubation later, the OD value was determined.

3. Result

The MTT method results showed that the cytotoxicity ratio of the 100% test article extract was 72.4%. The results of control groups showed the test was valid.

4. Conclusion

Under the conditions of this study, the test article extract did not show potential toxicity to L-929 cells.

1. STUDY SUMMARIES

1.1. Purpose

The purpose of the test is to determine the biological reactivity of a mammalian cell culture (mouse fibroblast L-929 cells) in response to the test article extract.

1.2. Referred Standard

- ISO 10993-5: 2009
Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity
- ISO 10993-12: 2012
Biological evaluation of medical devices - Part 12: Sample preparation and reference materials

1.3. Deviation(s) and Incident(s) Treatment

If any deviation or incident occurred during the test, the related information would be recorded timely and a deviation report should be submitted with the final report to interpretate the specific effect(s) on the final result caused by the deviation or incident.

1.4. Schedule of The Study

Technical Initiation Date: 2020-12-23

Technical Completion Date: 2020-12-25

2. TEST MATERIAL

2.1. General information¹⁾

Name: Oxford fabric
Initial state: Non-sterile
Size: N/S²⁾
Model: Black 600 D
Lot/ Batch#: N/S
Physical State: Solid
Color: N/S
Density: N/S
Stability: N/S
Solubility: N/S
Storage Condition: Room temperature
Test Article Material: N/S
Packaging Material: N/S
Expiry Date: N/S
Manufacturer Name: N/S
Manufacturer Address: N/S

- 1) The information about the test article was supplied by the sponsor wherever applicable.
2) N/S means not supplied by the sponsor.

2.2. Blank Control

Name: MEM medium with 10% FBS
Manufacturer: Hyclone
Size: 500mL
Lot/ Batch#: AF29549370
Physical State: Liquid
Color: Pink
Storage Condition: (2~8)°C

2.3. Negative Control

Name: High Density Polyethylene
Manufacturer: U.S.Pharmacopeia
Size: 3 Strips
Lot/ Batch#: KOM357
Physical State: Solid

Color: White
Storage Condition: Room temperature
Extract ratio: 3cm²:1mL
Extract Condition: 37°C, 24 h

2.4. Positive Control

Name: Zinc diethyldithiocarbamate
Manufacturer: Aladdin
Size: 25g
Lot/ Batch#: K1301032
Physical State: Solid
Color: White
Storage Condition: (2~8) °C
Final Concentration: 1%
Extract Condition: 37°C, 24 h

2.5. Main Instruments and Reagents

2.5.1 Instruments

Name	No.	Calibration Due Date
CO ₂ Incubator	EPB-122	2021-09-28
Shaking Water Bath	EPB-232	2021-09-28
Inverted Microscope	EPB-003	2021-09-28
Micro-plate Reader	EPB-192	2021-09-28

2.5.2. Main reagents

Name	Lot/ Batch#	Source
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT)	C10769615	PERFEMIKER
Fetal bovine serum (FBS)	2064652	Gibco
Minimum essential medium (MEM)	AF29549370	Hyclone
Penicillin streptomycin sulfate	J190044	Hyclone
Trypsin	J200009	Hyclone
Isopropanol	20200313	SCR

3. JUSTIFICATION OF THE TEST SYSTEM

Historically, mouse fibroblast L-929 cells have been used for cytotoxicity studies because they demonstrate sensitivity to extractable cytotoxic articles.

4. IDENTIFICATION OF TEST SYSTEM

L-929 mouse fibroblast cells obtained from Shanghai Cell Line Resources, CAS.

5. ROUTE OF ADMINISTRATION

The test article was extracted and administrated in vitro to mouse fibroblast L-929 cells through a solvent compatible with the test system. This was the optimal route of administration available in this test system as recommended in the guidelines.

6. TEST DESIGN

6.1. Extract Preparation

Table 1 Extraction process

Aseptically Sampling			Solvent	Sterilization Method	Extract Condition
Ratio	Sampling Manner	Actually Sampling			
0.1g/1mL	Random	0.91g	MEM with 10% FBS	UV	37°C 24 h

Table 2 Final extract¹⁾

Extract	pH	Clear or Not	Color changed or not	Presence of particles or not	Diluted/ filtered/pH adjusted or not
9.10 mL	7.5	Clear	Not	Not	Not

1) The final extracts used immediately.

6.2. MTT Solution Preparation

100 mg MTT powder was soluted in 100 mL PBS. Then sterilized by filtering with 0.22 µm membrane and kept in the dark at 4°C for one week.

6.3. Experimental Process

Aseptic procedures were used for handling of cell cultures.

L-929 cells were cultured in MEM medium (10% FBS, Penicillin 100 U/mL, Streptomycin 100 µg/mL) at 37°C in a humidified atmosphere of 5% CO₂, then digested with 0.25% trypsin to get single cell suspension. And obtained a 1×10⁵ cells/mL suspension by centrifuging (1000 rpm, 5 min) and re-suspended in MEM medium finally.

The suspended cells were dispensed at 100 µL per well in 96-well plate, and cultured it in cell incubator (5% CO₂, 37°C), Cell morphology was evaluated to verify that the monolayer was satisfactory.

After the cells grew to form a monolayer, original culture medium was discarded. The 96-well plates were then treated with 100 µL extract of test article (100%, 75%, 50%, 25%), blank control, negative control (100%) and positive control (100%) respectively. Incubated the 96-well plate incubator (37°C, 5%CO₂) for another 24h. Six parallel wells of each test were tested.

After 24h incubation, firstly observed the cell morphology and then discarded the culture medium. 50 µL aliquot of MTT (1 mg/mL) was added to each well and then incubated at 37°C in a humidified atmosphere of 5% CO₂ for 2h. The liquid in each well was tipped out and 100 µL isopropanol was added to each well. Evaluated the OD Value with a dual-wavelength spectrophotometer with the measurement wavelength at 570 nm and reference wavelength at 650 nm.

7. DATA ANALYSIS

Mean±standard deviation($\bar{X} \pm SD$)

$$\text{Viab.\%} = \frac{100 \times OD_{570e}}{OD_{570b}}$$

OD_{570e}—is the mean value of the measured optical density of the extracts of the test article;

OD_{570b}—is the mean value of the measured optical density of the blanks.

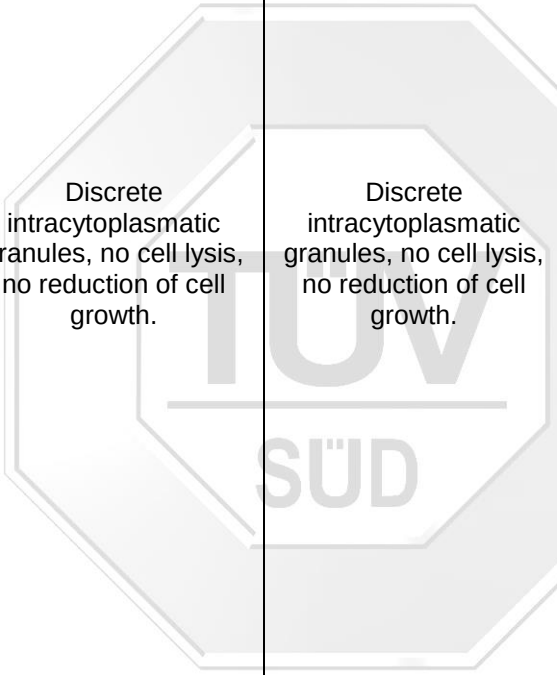
8. EVALUATION CRITERION

- The lower the Viab.% value, the higher the cytotoxic potential of the test article is.
- If Viab.% is reduced to <70% of the blank, it has a cytotoxic potential.

9. RESULTS

9.1 Results of Cell Morphology

Table 3 Observation of cell morphology

Group	Before inoculation	Before treated with extract	24h after treatment
Blank control	 Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
Negative control			Not more than 20% of the cells are round, loosely attached and without intracytoplasmatic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
Positive control			Nearly complete or complete destruction of the cell layers.
100% Test article extract			Not more than 50% of the cells are round, devoid of intracytoplasmatic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
75% Test article extract			Not more than 20% of the cells are round, loosely attached and without intracytoplasmatic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
50% Test article extract			Not more than 20% of the cells are round, loosely attached and without intracytoplasmatic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
25% Test article extract			Not more than 20% of the cells are round, loosely attached and without intracytoplasmatic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.

9.2 Results of Cell Vitality

Table 4 Results of cell vitality

Group	$\bar{X} \pm SD$	Viability%
Blank control	0.504±0.025	100.0%
Negative control	0.441±0.034	87.5%
Positive control	0.016±0.002	3.2%
100% test article extract	0.365±0.012	72.4%
75% test article extract	0.448±0.048	88.9%
50% test article extract	0.459±0.033	91.1%
25% test article extract	0.493±0.026	97.8%

10. CONCLUSION

Under the conditions of this study, the test article Oxford fabric extract did not show potential toxicity to L-929 cells.

Note:

- 1.This test was carried out by external laboratory assessed as competent.
- 2.This report is for internal use only such as internal scientific research ,education, quality control, product R&D.

-END OF THE TEST REPORT-