Safety Report

FluoroVue™ Nucleic Acid Gel Stain (NS1000)

Test System

The cytotoxicity test is performed by Super Laboratory Co., Ltd., New Taipei City, Taiwan (Report No. M62-180500005001) according to the requirements described in Biological evaluation of medical devices – Part 5: Tests for *in vitro* cytotoxicity (ANSI/AAMI/ISO 10993-5).

Purpose

The cytotoxicity test is designed to evaluate the acute adverse biological effects of chemical compounds or extractable from medical device materials. Cytotoxicity is preferred as a pilot project test and an important indicator for toxicity evaluation as it is simple, fast, has a high sensitivity and can save animals from toxicity.

Materials and Methods

- **Test Substance:**
  - Chemical name: FluoroVue™ Nucleic Acid Gel Stain (SMOBIO, NS1000)

- **Cell line and culture condition:**
  - Cell line: L-929 cell (NCTC clone 929, BCRC RM60091)
  - Culture Medium: Eagle's minimum essential medium (MEM) containing 10 % fetal bovine serum (FBS) and 2.0 mM L-Glutamine
  - Culture condition: 37°C ± 1°C, 5 ± 1% CO_2

- **Treatment group:**
  - Negative control: Minimum Essential Medium (GIBCO) with 10% FBS (NQBB)
  - Positive control: phenol (2 µl/ml, SIGMA-ALDRICH)
  - Test item: FluoroVue™ Nucleic Acid Gel Stain (1X working concentration, SMOBIO, NS1000)

- **Methods:**
  - The *in vitro* cytotoxicity test method was performed for the given test sample as pre ISO 10993-5, 2009.
  - L-929 cells were treated with the test item, negative control or positive control. Triplicate plates are prepared for each treatment.
  - Morphologic qualitative analysis: The cells were incubated for 24 hours and observed microscopically for cytotoxic effects. Cultures were observed under microscopy and graded for reactivity using a 0 to 4 scale. Definition of score values (based on ISO 10993-5 : 2009):

0 = no reactivity	Discrete intracytoplasmatic granules; no cell lysis
1 = slight reactivity	Not more than 20 % of the cells are round, loosely attached, and without intracytoplasmatic granules; occasional lysed cells are present
2 = mild reactivity	Not more than 50 % of the cells are round and devoid of intracyto-plasmatic granules; no extensive cell lysis and empty areas between cells
3 = moderate reactivity	Not more than 70 % of the cell layers contain rounded cells or are lysed
4 = severe reactivity	Nearly complete destruction of the cell layers

  - MTT quantitative analysis: The culture medium from the L929 cells was replaced with culture medium containing the test item, negative control or positive control. After incubation for 24 hrs, MTT were added in all the wells and incubated for 2 ± 0.5 hrs. After incubation, DMSO were added in the wells and read at 570 nm using spectrophotometer. Mean value of growth inhibition was calculated by the formula as below.
    Mean value growth inhibition = 100% x $A570_{(Negative\ control)}$ – $A570_{(Positive\ control\ or\ Test\ item)}$/ $A570_{(Negative\ control)}$

Results

– Qualitative analysis: Results of morphology of cells after 24 hrs treatment

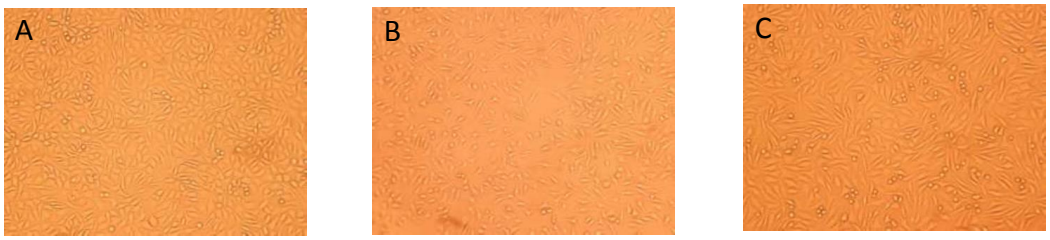

Figure 1. Morphology of cells after 24 hrs treatment. (A) Negative control, culture medium contained 10% FBS. (B) Positive control, culture medium contained phenol. (C) Test item, culture medium contained FluoroVue™ Nucleic Acid Gel Stain. Cells treated with negative control and test item displayed no lysis. Nearly complete destruction of the cell layers was observed in cells treated with positive control.

Table 1. Results of microscopical evaluation

Treatment Group	Treatment duration (hr)	Morphology of cells[a]	Score values[b]
Negative control[c]	24 ± 1	Discrete intracytoplasmic granules, no cell lysis.	0
Positive control[d]	24 ± 1	Nearly complete destruction of the cell layers.	4
Test item[e]	24 ± 1	Discrete intracytoplasmic granules, no cell lysis.	0

[a] Triplicate experiments were analyzed for each treatment.
[b] Definition of Score value based on ISO10993-5 : 2009
[c] culture medium contained 10% FBS
[d] culture medium contained phenol
[e] culture medium contained FluoroVue™ Nucleic Acid Gel Stain

– Results of growth inhibition

Table 2. Results of MTT quantitative analysis

Treatment Group	Absorbance (570 nm)[a]	Mean value growth inhibition [%][b]
Negative control[c]	1.136 ± 0.024	0.0
Positive control[d]	0.028 ± 0.001*	97.5
Test item[e]	1.049 ± 0.015*	7.6

[a] Triplicate experiments were analyzed for each treatment, absorbance results were shown in Mean ± SD
[b] Mean value growth inhibition = 100% x $A570_{(Negative\ control)} - A570_{(Positive\ control\ or\ Test\ item)} / A570_{(Negative\ control)}$
If the mean value of test item was less than 0%, data is presented as 0%.
[c] culture medium contained 10% FBS
[d] culture medium contained phenol
[e] culture medium contained FluoroVue™ Nucleic Acid Gel Stain
* Significant different to negative control group (One-Way ANOVA, p <0.05).

Conclusion

Due to the high sensitivity of the mouse fibroblast growth inhibition test, it is assumed that a mean growth inhibition of up to 30 % does not indicate a significant risk of cytotoxicity. Based upon the observed results and under the test-conditions chosen, the test substance "FluoroVue™ Nucleic Acid Gel Stain (SMOBIO, NS1000)" is considered to have no cytotoxic effects since the grade was zero in microscopical evaluation and mean growth inhibition was 7.6% in the growth inhibition test with L929 mouse fibroblasts.

References

1. International Organization for Standardization (ISO). Biological evaluation of medical devices-part 5: Test for *in vitro* cytotoxicity, ISO10993-5, 2009
2. International Organization for Standardization (ISO). Biological evaluation of medical devices-part 12: Sample preparation and reference materials, ISO10993-12, 2012