SERVA HiSens Stain G Cat. no. 39805

1. Introduction

Ethidium bromide (EtBr) is most commonly used nucleic acid stain in molecular biology laboratories. It has been proved to be strong carcinogen and therefore considered hazardous for laboratory personnel and environment.

SERVA HiSens Stain G is a nucleic acid stain specially designed for in-gel use and is a safer replacement for conventional Ethidium bromide (EtBr), which poses a significant health and safety hazard for its user. It is a fluorescent stain which offers high sensitivity detection of double-stranded or single-stranded DNA and RNA in a convenient manner.

SERVA HiSens Stain G offers high sensitivity that is several times greater than EtBr.

The safety of **SERVA HiSens Stain G** has been controlled with following tests:

- Cytotoxicity Test
- Ames Test

2. Safety Tests

2.1. Cytotoxicity Test

2.1.1. Test System

The cytotoxicity test is performed according to the requirements described in Biological evaluation of medical devices – Part 5: Tests for in vitro cytotoxicity (ANSI/AAMI/ISO 10993-5).

2.1.2. 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.

2.1.3. Materials and Methods

Test Substance

Chemical name: **SERVA HiSens Stain G** (cat. no. 39805)

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: **SERVA HiSens Stain G**; 10,000-fold diluted

Methods

The *in vitro* cytotoxicity test method was performed for the given test sample as per 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):

Score	Reactivity	Effects
0	**No**	Discrete intra-cytoplasmatic granules; no cell lysis
1	**Slight**	Not more than 20 % of the cells are round, loosely attached, and without intra-cytoplasmatic granules; occasional lysed cells are present
2	**Mild**	Not more than 50 % of the cells are round and devoid of intra-cytoplasmatic granules; no extensive cell lysis and empty areas between cells
3	**Moderate**	Not more than 70 % of the cell layers contain rounded cells or are lysed
4	**Severe**	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 24 h incubation, MTT was added in all the wells and incubated for 2 ± 0.5 h.
Then, DMSO was added in each well and analysed at 570 nm using spectrophotometer.

Mean value of growth inhibition was calculated by the formula as below.

Mean value growth inhibition = 100 % x A_{570} (negative control) – A_{570} (positive control or test item)/ A_{570} (negative control)

2.1.4. Results

- Cell Morphology

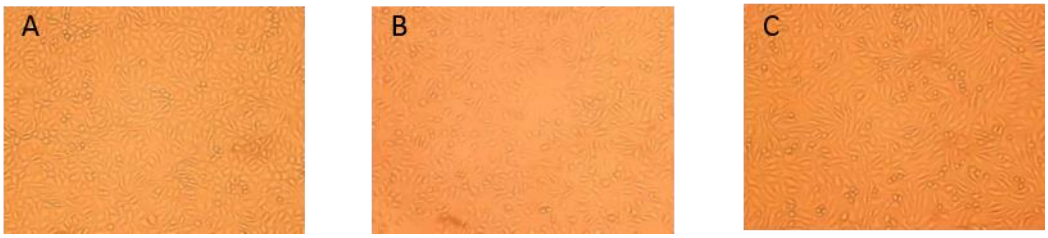

Figure 1: Morphology of cells after 24 hrs treatment.

(A) Negative control, culture medium contained 10% FBS.
(B) Positive control, culture medium contained phenol.
(C) Test item, culture medium contained 10,000-fold diluted test substance.
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.

Treatment group	Treatment duration [h]	Cell morphology [a]	Score value [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

Table 1: Results of microscopical evaluation

[a] Experiments were performed as triplicates and analysed for each treatment.
[b] Definition of Score value based on ISO10993-5:2009
[c] Culture medium containing 10 (v/v) % FBS
[d] Culture medium containing phenol (2 µl/ml)
[e] Culture medium containing 10,000-fold diluted test substance

- Growth Inhibition

Treatment group	Absorbance [570 nm] [a]	Mean value of 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**

Table 2: Results of MTT quantitative analysis

[a] Experiments were performed as triplicates and analysed for each treatment, absorbance results are 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 the test item is below 0 %, data is presented as 0 %.
[c] Culture medium containing 10 (v/v) % FBS
[d] Culture medium containing phenol (2 µl/ml)
[e] Culture medium containing 10,000-fold diluted test substance

2.1.5. 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 **SERVA HiSens Stain G** (SERVA cat. no. 39805) 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.

2.1.6. References

- International Organization for Standardization (ISO). Biological evaluation of medical devices-part 5: Test for in vitro cytotoxicity, ISO10993-5, 2009.

- International Organization for Standardization (ISO). Biological evaluation of medical devices-part 12: Sample preparation and reference materials, ISO10993-12, 2012.

2.2. Ames Test

2.2.1. Test system

The Ames Test study is conducted according to the Organization for Economic Co-operation and Development (OECD) guideline for testing of chemicals, bacterial reverse mutation test (OECD 471, 1997).

2.2.2. Purpose

The Ames test is a standard assay to assess the mutagenic potential of chemicals.
As cancers are often associated with DNA damage, this test can be used to estimate the carcinogenic potential of a chemical compound.

2.2.3. Material and Methods

Test Substance

Chemical name: **SERVA HiSens Stain G** (cat. no. 39805)

Tested concentration: 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml, 1 mg/ml, and 2 mg/ml
[Final concentration: 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml]

Bacterial Strains

Salmonella Typhimurium, TA98 and TA100

Methods

The following was added to each sterile culture tube containing 2.0 mL top agar:
The 0.1 mL of test substance, 0.1 mL of bacterial suspension, and 0.5 mL of S9 mixture (+S9) or 0.5 mL of without S9 mixture (-S9).

The mixture was uniformly poured on the prepared underlay agar plates. After solidification, the plates were incubated at 37 °C for 48 h. At the end of the incubation, revertant colonies per plate were counted. All plating was done in triplicate. If the number was more than twice the spontaneous revertant colonies counts and showed a dose-response relationship, positive result for mutagenicity could be concluded.

2.2.4. Results

According to the results of the Ames test, in the presence and absence of metabolic activator S9 the increase in the numbers of revertant colonies of strains TA98, and TA100 compared to spontaneous revertant colonies was less than 2 times, and there was no dose-response relationship. Appropriate reference mutagens and Ethidium Bromide were used as positive controls and they showed a distinct increase of induced revertant colonies.

Treatment	Revertant colonies [CFU/plate]			
	TA98		TA100	
	- S9	+ S9	- S9	+ S9
Negative control (H_2O)	20 ± 2	20 ± 4	74.67 ± 2.31	92 ± 4.58
SERVA HiSens Stain G				
6.25 µg/ml	16 ± 3	22 ± 4	67.33 ± 3.79	20 ± 2
12.5 µg/ml	25 ± 3	20 ± 0	66.67 ± 0.58	20 ± 2
25 µg/ml	23 ± 1	27 ± 6	74 ± 7	20 ± 2
50 µg/ml	16 ± 0	22 ± 1	77.33 ± 4.04	20 ± 2
100 µg/ml	22 ± 2	20 ± 1	80.33 ± 9.29	20 ± 2
EtBr – 4 µg/ml	18 ± 2	469 ± 11*	106.67 ± 8.62	119.67 ± 3.21
Positive control	2-NF (2 µg)	2-AA (5 µg)	NaN_3 (5 µg)	2-Anthramine (5 µg)
	494 ± 47	674 ± 86	917.67 ± 101.24*	1809.67 ± 196.51*

2.2.5. Conclusion

According to the guidelines, negative result was obtained and a significant mutagenic effect of **SERVA HiSens Stain G** (SERVA cat. no. 39805) could not be detected.

2.2.6. References

- Organization for Economic Co-operation and Development (OECD). OECD Guideline for Testing of Chemicals, Bacterial Reverse Mutation Test, OECD 471, 1997.