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WUXI APPTEC (SUZHOU) CO., LTD.

Test Report

Sample Name: Nucleic acid gel electrophoresis reagent (Blue Ray)

Manufacture: UElandy (Suzhou) Co., Ltd.

Test Item: Mini-Ames Assay

Date Issued: 2021-09-15

Sample Name:	Nucleic acid gel electrophoresis reagent (Blue Ray)		
Batch No.:	HS1009	Amount:	2.0 mL
Test Item:	Mini-Ames Assay	Receipt Time:	2021-02-24
Manufacture:	UElandy (Suzhou) Co., Ltd.		
Tel:	18013768787		
First Dosing Date:	2021-03-05	Final Report Date:	2021-08-11

Nucleic acid gel electrophoresis reagent (Blue Ray): Mini-Ames Assay

Summary

The Mini-Ames assay was conducted in the presence and absence of exogenous metabolic activation (Aroclor 1254 induced rat liver S9), along with concurrent negative/solvent control (DMSO) using six wells and positive controls using three wells. Nucleic acid gel electrophoresis reagent (Blue Ray) did not induce more than 2-fold increase in strains TA98, TA100, or WP2 *uvr*A (pKM101), nor 3-fold increase in strains TA1535 or TA1537 in the mean number of revertant colonies at any dose level relative to the concurrent negative/solvent control, either in the presence or absence of the S9 mix. No dose response was observed either. It was concluded that Nucleic acid gel electrophoresis reagent (Blue Ray) was negative for mutagenicity under the conditions of this study.

1. OBJECTIVE

The objective of this Mini-Ames study was to evaluate the test article Nucleic acid gel electrophoresis reagent (Blue Ray) by measuring its ability to induce reverse mutations both in the presence and absence of mammalian microsomal enzymes at the histidine locus in the genome of four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and at the tryptophan locus in the genome of *Escherichia coli* WP2 *uvr*A (pKM101).

2. Test Article Characterization and Dose Formulation Preparation

The test article Nucleic acid gel electrophoresis reagent (Blue Ray) was liquid, stored at room temperature and protected form light. In this study, lower concentrations were obtained by serial dilution with the DMSO (dimethyl sulfoxide).

3. Tester Strains

Salmonella typhimurium histidine auxotrophs TA98, TA100, TA1535, TA1537 and *Escherichia coli* tryptophan auxotroph strain WP2 *uvr*A (pKM101) were purchased from Molecular Toxicology (Boone, NC). The genotypes of all tester strains used in this assay were confirmed.

4. Main reagent

Positive control articles: 2-Aminoanthracene [2-AA, CAS 613-13-8]; 2-Nitrofluorene [2-NF, CAS 607-57-8]; Sodium Azide [SA, CAS 26628-22-8]; Acridine mutagen ICR-191 [ICR-191, CAS 17070-45-0]; N-Methyl-N-nitro-N-nitrosoguanidine [MNNG, CAS 70-25-7].

S9 homogenate was purchased from Molecular Toxicology (Boone, NC) and stored frozen at -80°C freezer until use.

5. Dose Levels Design

Table I Negative/Solvent Control Group

Treatment	S9 Activation	Tester Strains	Dose Volume
DMSO	±	TA98, TA100, TA1535, TA1537, and WP2 *uvr*A (pKM101)	20 μL/well

Table II Positive Controls Groups

Treatment	S9 Activation	Tester Strains	Dose Concentration	Dose Levels
2-Aminoanthracene	+	TA98, TA100, TA1535 and TA1537	20 μg/mL	0.4 μg/well
2-Aminoanthracene	+	WP2 *uvr*A (pKM101)	100 μg/mL	2.0 μg/well
2-Nitrofluorene	-	TA98	100 μg/mL	2.0 μg/well
Sodium Azide	-	TA100 and TA1535	10 μg/mL	0.2 μg/well
ICR-191	-	TA1537	10 μg/mL	0.2 μg/well
MNNG	-	WP2 *uvr*A (pKM101)	10 μg/mL	0.2 μg/well

Table III Test Article Groups

Treatment	S9 Activation	Tester Strains	Dose Concentration	Dose Levels
Nucleic acid gel electrophoresis reagent (Blue Ray)	±	TA98, TA100, TA1535, TA1537, and WP2 *uvr*A (pKM101)	50, 20, 8, 3.2, 1.25, 0.5, 0.2, and 0.075 mg/mL	1000, 400, 160, 64, 25, 10, 4, and 1.5 μg/well

6. Treatment of Test System

A top agar consisting of 0.6% (w/v) agar and 0.5% (w/v) NaCl was melted. A solution of 0.5 mM L-histidine/biotin or 0.5 mM L-tryptophan solution was added to the melted top agar at a ratio of 10 mL per 100 mL top agar, this solution was aliquoted at 1.6 mL per tube and held at approximately 45°C±2°C. Then 80 μL of the test/control article solutions, 400 μL of S9 mix (for S9 activated) or phosphate buffered saline (for non-activated) and 80 μL of the bacterial cultures were added to the 1.6 mL of top agar. After mixing the top agar mix, every 540 μL of the resulting mix was added into one well of the six-well plates (34.8-mm dishes) containing solidified approximately 5 mL of minimal glucose agar media (1.5% agar, 2% glucose, in Vogel-Bonner medium E).

Each treatment was plated in triplicate (three wells) except for solvent/negative (DMSO) controls which were plated in sextuplicate (six wells).

As soon as the soft agar solidified, the six-well plates were incubated at 37°C±2°C for about 68 hours.

7. Evaluation of Test Results

The Mini-Ames assay must be determined to be valid before final evaluations are made. Once the criteria for a valid assay are met, responses observed in the assay are evaluated as follows. In addition to the criteria below, biological relevance is also taken into

account, for example the historical negative control range and consistency of response within and between concentrations and (where applicable) between experiments. The final evaluation of the test article is based on scientific judgment.

Positive Response

For a test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per well of at least one tester strain over a minimum of two increasing concentrations of test article as specified below:

- Strains TA98, TA100, and WP2 *uvr*A (pKM101)

Data sets are judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 2.0-fold the mean solvent control value.

- Strains TA1535 and TA1537

Data sets are judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 3.0-fold the mean solvent control value.

Equivocal

An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-related increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited.

Negative Response

A test article is evaluated negative, if none of the above criteria are met.

8. RESULTS

Nucleic acid gel electrophoresis reagent (Blue Ray) did not induce more than 2-fold increase in strains TA98, TA100, or WP2 *uvr*A (pKM101), nor 3-fold increase in strains TA1535 or TA1537 in the mean number of revertant colonies at any dose level when compared to the concurrent negative/solvent (DMSO) control, either in the presence or absence of the S9 mix. No dose response was observed either.

9. CONCLUSION

The results of the Mini-Ames assay indicated that under the conditions of the study, the Nucleic acid gel electrophoresis reagent (Blue Ray) was concluded to be negative.