

WUXI APPTEC (SUZHOU) CO., LTD.



Test Report

Sample Name: Super GelGreen (GelBlue) II 5000×

Supplier: Yuheng (Suzhou) Biotechnology Co., Ltd.

Test Item: Mini-Ames Assay

Date Issued: 2019-10-14



Sample Name: Super GelGreen (GelBlue) II 5000×
Lot/Batch No.: HS0820 Amount: 1.0 mL
Test Item: Mini-Ames Assay Receipt Time: 2019-08-21
Supplier: Yuheng (Suzhou) Biotechnology Co., Ltd.
Address: Room C-1003, NO.1 Guantang Road, Xiangcheng District, Suzhou
P.R., China
Tel: 0512-69571710 Website: www.useverbright.com
First Dosing Date: 2019-08-29 Final Report Date: 2019-10-14

Super GelGreen (GelBlue) II 5000×: 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. Super GelGreen (GelBlue) II 5000× did not induce more than 2-fold increase in strains TA98, TA100, or WP2 *uvrA* (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 Super GelGreen (GelBlue) II 5000× 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 Super GelGreen (GelBlue) II 5000× 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 *uvrA* (pKM101).

2. Test Article Characterization And Dose Formulation Preparation

The test article Super GelGreen (GelBlue) II 5000× was orange liquid, and stored at room temperature. In this study, the concentration of the test article is 100 mg/mL provided by the Sponsor. Lower concentrations were obtained by serial dilution with the DMSO (dimethyl sulfoxide).

3. Strain Source and Genotypic Characterization

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

4. Main reagent

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

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 <i>uvrA</i> (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 <i>uvrA</i> (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 <i>uvrA</i> (pKM101)	10 µg/mL	0.2 µg/well

Table III Test Article Groups

Treatment	S9 Activation	Tester Strains	Dose Concentration	Dose Levels
Super GelGreen (GelBlue) II 5000×	±	TA98, TA100, TA1535, TA1537, and WP2 <i>uvrA</i> (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 67.5 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 *uvrA* (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

Super GelGreen (GelBlue) II 5000× did not induce more than 2-fold increase in strains TA98, TA100, or WP2 *uvrA* (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 Super GelGreen (GelBlue) II 5000× was concluded to be negative.