

# ACB Cationic Glycoprotein PF Toxicology Data

**Code:** 20391PF  
**INCI Name:** Lactobacillus/Eriodictyon Californicum Ferment Extract & Phospholipids  
**CAS #:** 68990-14-7 & 8002-43-5  
**EINECS #:** 273-580-8 & 232-207-2

Name of Study	Type of Study	Results
Dermal & Ocular Irritation Tests	<i>In-vitro</i>	Both the dermal and ocular assays reveal that <b>ACB Cationic Glycoprotein PF</b> is non-irritating and should not cause any of the aforementioned conditions.
AMES Test	<i>In-vitro</i>	The results of the Bacterial Reverse Mutation Assay indicate that under the conditions of this assay, that <b>ACB Cationic Glycoprotein PF</b> was considered to be Non-Mutagenic to Salmonella typhimurium testerstrains TA98, TA100, TA1537, TA1535 and Escherichia coli WP2uvrA



# Dermal and Ocular Irritation Tests

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**Sample:** ACB Cationic Glycoprotein PF

**Code:** 20391PF

**CAS #:** 68990-14-7 & 8002-43-5

**Test Request Form/Submission #:** 117

**Lot #:** 25561

**Sponsor:** Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092

**Study Director:** Erica Segura

**Principle Investigator:** Meghan Darley

**Test Performed:**

In Vitro EpiDerm™ Dermal Irritation Test (EPI-200-SIT)

EpiOcular™ Eye Irritation Test (OCL-200-EIT)

## **SUMMARY**

*In vitro* dermal and ocular irritation studies were conducted to evaluate whether **ACB Cationic Glycoprotein PF** would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays.

The product was tested according to the manufacture's protocol. The test article solution was found to be **non-irritating**. Reconstructed human epidermis and cornea epithelial model were incubated in growth media overnight to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substances were applied to the tissue inserts and incubated for 60 minutes for liquid and solid substances in the EpiDerm™ assay and 30 minutes for liquid substances and 90 minutes for solid substances in the EpiOcular™ assay at 37°C, 5% CO<sub>2</sub>, and 95% relative humidity (RH). Tissue inserts were thoroughly washed and transferred to fresh plates with growth media. After post substance dosing incubation is complete, the cell viability test begins. Cell viability is measured by dehydrogenase conversion of MTT [(3-4,5-dimethyl thiazole 2-yl)], present in the cell mitochondria, into blue formazan salt that is measured after extraction from the tissue. The irritation potential of the test chemical is dictated by the reduction in tissue viability of exposed tissues compared to the negative control.

Under the conditions of this assay, the test article was considered to be **non-irritant**. The negative and positive controls performed as anticipated.

## **I. Introduction**

### **A. Purpose**

*In vitro* dermal and ocular irritation studies were conducted to evaluate whether a test article would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays. MatTek Corporation's reconstructed human epidermal and human ocular models are becoming a standard in determining the irritancy potential of test substances. They are able to discriminate between irritants and non-irritants. The EpiDerm™ assay has accuracy for the prediction of UN GHS R38 skin irritating and no-label (non-skin irritating) test substances. The EpiOcular™ assay can differentiate chemicals that have been classified as R36 or R41 from the EU classifications based on Dangerous Substances Directive (DSD) or between the UN GHS Cat 1 and Cat 2 classifications.

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# Dermal and Ocular Irritation Tests

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## II. Materials

- A. Incubation Conditions:** 37°C at 5% CO<sub>2</sub> and 95% relative humidity
- B. Equipment:** Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; Pipettes
- C. Media/Buffers:** DMEM based medium; DPBS; sterile deionized H<sub>2</sub>O
- D. Preparation:** Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components
- E. Tissue Culture Plates:** Falcon flat bottom 96-well, 24-well, 12-well, and 6-well tissue culture plates
- F. Reagents:** MTT (1.0mg/mL); Extraction Solution (Isopropanol); SDS (5%); Methyl Acetate
- G. Other:** Nylon Mesh Circles (EPI-MESH); Cotton tip swabs; 1mL tuberculin syringes; Ted Pella micro-spatula; 220mL specimen containers; sterile disposable pipette tips; Parafilm

## III. Test Assay

### **A. Test System**

The reconstructed human epidermal model, EpiDerm™, and cornea epithelial model, EpiOcular™, consist of normal human-derived epidermal keratinocytes which have been cultured to form a multilayer, highly differentiated model of the human epidermis and cornea epithelium. These models consist of organized basal, spinous, and granular layers, and the EpiDerm™ systems also contains a multilayer stratum corneum containing intercellular lamellar lipid layers that the EpiOcular™ system is lacking. Both the EpiDerm™ and EpiOcular™ tissues are cultured on specially prepared cell culture inserts.

### **B. Negative Control**

Sterile DPBS and sterile deionized water are used as negative controls for the EpiDerm™ and EpiOcular™ assays, respectfully.

### **C. Positive Control**

Known dermal and eye irritants, 5% SDS solution and Methyl Acetate, were used as positive controls for the EpiDerm™ and EpiOcular™ assays, respectfully.

### **D. Data Interpretation Procedure**

#### **a. EpiDerm™**

An irritant is predicted if the mean relative tissue viability of the 3 tissues exposed to the test substance is reduced by 50% of the mean viability of the negative controls and a non-irritant's viability is > 50%.

#### **b. EpiOcular™**

An irritant is predicted if the mean relative tissue viability of the 2 tissues exposed to the test substance is reduced by 60% of the mean viability of the negative controls and a non-irritant's viability is > 40%.

## IV. Method

### **A. Tissue Conditioning**

Upon MatTek kit arrival at Active Concepts, LLC the tissue inserts are removed from their shipping medium and transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO<sub>2</sub> and 95% relative humidity for 60 minutes. After those 60 minutes the inserts are transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO<sub>2</sub> and 95% relative humidity for an additional 18 to 21 hours.

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## B. Test Substance Exposure

### a. EpiDerm™

30µL (liquid) or 25mg (solid) of the undiluted test substance is applied to 3 tissue inserts and allowed to incubate for 60 minutes in a humidified incubator (37°C, 5% CO<sub>2</sub>, 95% RH).

### b. EpiOcular™

Each tissue is dosed with 20µL DPBS prior to test substance dosing. 50µL (liquid) or 50mg (solid) of the undiluted test substance is applied to 2 tissue inserts and allowed to incubate for 90 minutes in a humidified incubator (37°C, 5% CO<sub>2</sub>, 95% RH).

## C. Tissue Washing and Post Incubation

### a. EpiDerm™

All tissue inserts are washed with DPBS, dried with cotton tipped swab, and transferred to fresh media and culture plates. After 24 hours the inserts are again transferred into fresh media and culture plates for an additional 18 to 20 hours.

### b. EpiOcular™

Tissue inserts are washed with DPBS and immediately transferred into 5mL of assay medium for 12 to 14 minutes. After this soak the inserts are transferred into fresh media and tissue culture plates for 120 minutes for liquid substances and 18 hours for solid substances.

## D. MTT Assay

Tissue inserts are transferred into 300µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% CO<sub>2</sub>, and 95% RH. Inserts are then removed from the MTT medium and placed in 2mL of the extraction solution. The plate is sealed and incubated at room temperature in the dark for 24 hours. After extraction is complete the tissue inserts are pierced with forceps and 2 x 200µL aliquots of the blue formazan solution is transferred into a 96 well plate for Optical Density reading. The spectrophotometer reads the 96-well plate using a wavelength of 570 nm.

## V. Acceptance Criterion

### A. Negative Control

The results of this assay are acceptable if the mean negative control Optical Density (OD<sub>570</sub>) is  $\geq 1.0$  and  $\leq 2.5$  (EpiDerm™) or  $\geq 1.0$  and  $\leq 2.3$  (EpiOcular™).

### B. Positive Control

#### a. EpiDerm™

The assay meets the acceptance criterion if the mean viability of positive control tissues expressed as a % of the negative control is  $\leq 20\%$ .

#### b. EpiOcular™

The assay meets the acceptance criterion if the mean viability of positive control tissues is  $< 60\%$  of control viability.

### C. Standard Deviation

Since each irritancy potential is predicted from the mean viability of 3 tissues for EpiDerm™ and 2 tissues for EpiOcular™, the variability of the replicates should be  $< 18\%$  for EpiDerm™ and  $< 20\%$  EpiOcular™.

## VI. Results

### A. Tissue Characteristics

The tissue inserts included in the MatTek EpiDerm™ and EpiOcular™ assay kits were in good condition, intact, and viable.

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## B. Tissue Viability Assay

The results are summarized in Figure 1. In no case was the tissue viability  $\leq 50\%$  for EpiDerm™ or  $\leq 60\%$  for EpiOcular™ in the presence of the test substance. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited substantial loss of tissue viability and cell death.

## C. Test Validity

The data obtained from this study met criteria for a valid assay.

## VII. Conclusion

Under the conditions of this assay, the test article substance was considered to be **non-irritating**. The negative and positive controls performed as anticipated.

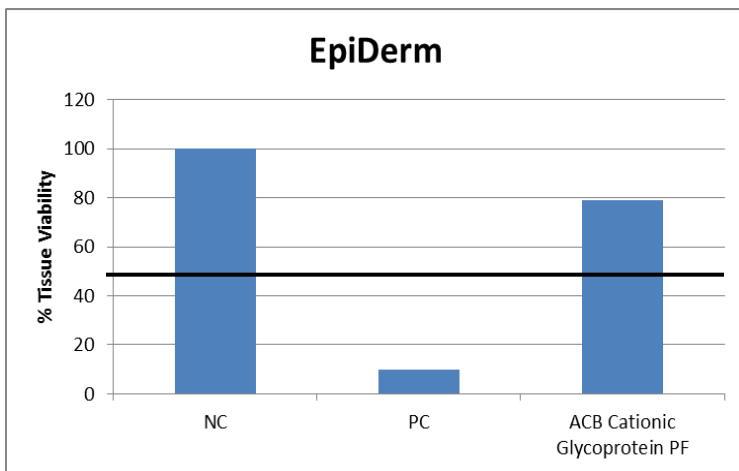


Figure 1: EpiDerm tissue viability

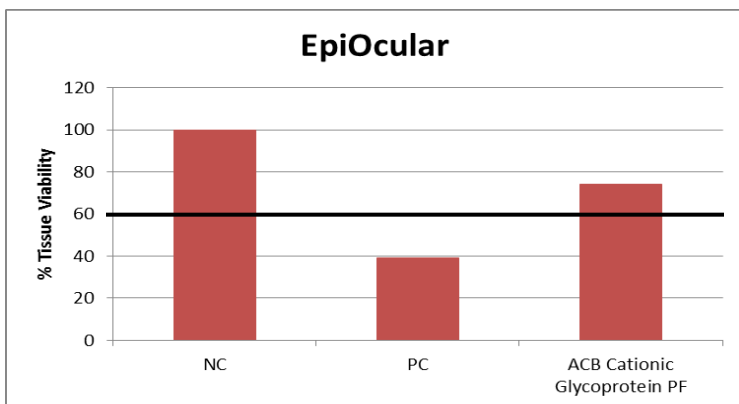


Figure 2: EpiOcular tissue viability



# Bacterial Reverse Mutation Test

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**Code Number:** 20391PF  
**CAS #:** 68990-14-7 & 8002-43-5

**Sponsor:**  
*Active Concepts, LLC*  
107 Technology Drive  
Lincolnton, NC 28092

**Study Director:** *Erica Segura*  
**Principle Investigator:** *Monica Beltran*

**Test Performed:**  
Genotoxicity: Bacterial Reverse Mutation Test

**Reference:**  
OECD471/ISO10993.Part 3

**Test Request Number:** 1626

## SUMMARY

A *Salmonella typhimurium*/*Escherichia coli* reverse mutation standard plate incorporation study described by Ames *et al.* (1975) was conducted to evaluate whether a test article solution **ACB Cationic Glycoprotein PF** would cause mutagenic changes in the average number of revertants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535 and tryptophan-dependent *Escherichia coli* strain WP2uvrA in the presence and absence of Aroclor-induced rat liver S9. This study was conducted to satisfy, in part, the Genotoxicity requirement of the International Organization for Standardization: Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

The stock test article was tested at eight doses levels along with appropriate vehicle control and positive controls with overnight cultures of tester strains. The test article solution was found to be noninhibitory to growth of tester strain TA98, TA100, TA1537, TA1535 and WP2uvrA after Spot Inhibition Screen.

Separate tubes containing 2 ml of molten top agar at 45°C supplemented with histidine-biotin solution for the *Salmonella typhimurium* strains and supplemented with tryptophan for *Escherichia coli* strain were inoculated with 100 µl of tester strains, 100 µl of vehicle or test article dilution were added and 500 µl aliquot of S9 homogenate, simulating metabolic activation, was added when necessary. After vortexing, the mixture was poured across the Minimal Glucose Agar (GMA) plates. Parallel testing was also conducted with positive control correspond to each strain, replacing the test article aliquot with 50µl aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for 48 hours at 37°C. The mean numbers of revertants of the test plates were compared to the mean number of revertants of the negative control plates for each of the strains tested. The means obtained for the positive controls were used as points of reference.

Under the conditions of this assay, the test article solution was considered to be Non-Mutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* tester strain WP2uvrA. The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 100993, Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicology.

## I. Introduction

### **A. Purpose**

A *Salmonella typhimurium*/*Escherichia coli* reverse mutation standard plate incorporation study was conducted to evaluate whether a test article solution would cause mutagenic changes in the average number of revertants for *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2uvrA in the presence and absences of the S9 metabolic activation. Bacterial reverse mutation tests have been widely used as rapid screening procedures for the determination of mutagenic and potential carcinogenic hazards.

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# Bacterial Reverse Mutation Test

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## II. Materials

- A. **Storage Conditions:** Room temperature (23-25C).
- B. **Vehicle:** Sterile DI Water.
- C. **Preparation:** Eight different doses level were prepared immediately before use with sterile DI water.
- D. **Solubility/Stability:** 100% Soluble and Stable.
- E. **Toxicity:** No significant inhibition was observed.

## III. Test System

### A. Test System

Each *Salmonella typhimurium* and *Escherichia coli* tester strain contains a specific deep rough mutation (*rfa*), the deletion of *uvrB* gene and the deletion in the *uvrA* gene that increase their ability to detect mutagens, respectively. These genetically altered *Salmonella typhimurium* strains (TA98, TA100, TA1537 and TA1535) and *Escherichia coli* strain (WP2*uvrA*) cannot grow in the absence of histidine and tryptophan, respectively. When placed in a histidine-tryptophan free medium, only those cells which mutate spontaneously back to their wild type states are able to form colonies. The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant, but if a mutagen is added to the test system, the mutation rate is significantly increased.

<u>Tester strain</u>	<u>Mutations/Genotypic Relevance</u>
TA98	hisD3052, Dgal chlD bio <i>uvrB rfa</i> pKM101
TA100	hisG46, Dgal chlD BIO <i>uvrB rfa</i> pKM101
TA1537	hisC3076, <i>rfa</i> , Dgal chlD bio <i>uvrB</i>
TA 1535	hisG46, Dgal chlD bio <i>uvrB rfa</i>
WP2 <i>uvrA</i>	trpE, <i>uvrA</i>

- rfa* = causes partial loss of the lip polysaccharide wall which increases permeability of the cell to large molecules.
- uvrB* = deficient DNA excision-repair system (i.e., ultraviolet sensitivity)
- pKM101 = plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens.
- uvrA* = All possible transitions and transversions, small deletions.

### B. Metabolic Activation

Aroclor induced rat liver (S9) homogenate was used as metabolic activation. The S9 homogenate is prepared from male Sprague Dawley rats. Material is supplied by MOLTOX, Molecular Toxicology, Inc.

### C. Preparation of Tester strains

Cultures of *Salmonella typhimurium* TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2*uvrA* were inoculated to individual flasks containing Oxoid broth No.2. The inoculated broth cultures were incubated at 37°C in an incubator shaker operating at 140-150 rpm for 12-16 hours.

### D. Negative Control

Sterile DI water (vehicle without test material) was tested with each tester strain to determine the spontaneous reversion rate. Each strain was tested with and without S9 activation. These data represented a base rate to which the number of revertants colonies that developed in each test plate were compared to determine whether the test material had significant mutagenic properties.

### E. Positive Control

A known mutagen for each strain was used as a positive control to demonstrate that tester strains were sensitive to mutation to the wild type state. The positive controls are tested with and without the presence of S9 homogenate.

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## F. Titer of the Strain Cultures:

Fresh cultures of bacteria were grown up to the late exponential or early stationary phase of growth; to confirm this, serial dilutions from each strain were conducted, indicating that the initial population was in the range of 1 to  $2 \times 10^9$ /ml.

## IV. Method

### A. Standard Plate Incorporation Assay:

Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the *Salmonella typhimurium* and tryptophan for *Escherichia coli* were inoculated with 100  $\mu$ l of culture for each strain and 100  $\mu$ l of testing solution or vehicle without test material. A 500  $\mu$ l aliquot of S9 homogenate, simulating metabolic activation, was added when necessary. The mixture was poured across Minimal Glucose Agar plates labeled with strain number and S9 activation (+/-). When plating the positive controls, the test article aliquot was replaced by 50  $\mu$ l aliquot of appropriate positive control. The test was conducted per duplicate. The plates were incubated for 37°C for 2 days. Following the incubation period, the revertant colonies on each plate were recorded. The mean number of revertants was determined. The mean numbers of revertants of the test plates were compared to the mean number of revertants of the negative control of each strain used.

## V. Evaluation

For the test solution to be evaluated as a test failure or "potential mutagen" there must have been a 2-fold or greater increase in the number of mean revertants over the means obtained from the negative control for any or all strains. Each positive control mean must have exhibited at least a 3-fold increase over the respective negative control mean of the *Salmonella* and *Escherichia coli* tester strain used.

## VI. Results and Discussion

### A. Solubility:

Water was used as a solvent. Solutions from the test article were made from 0.015 to 50mg/ml.

### B. Dose levels tested:

The maximum dose tested was 5000  $\mu$ g per plate. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000  $\mu$ g per plate.

### C. Titer (Organisms/ml):

$5 \times 10^8$  UFC/ml plate count indicates that the initial population was in the range of 1 to  $2 \times 10^9$  UFC/ml.

### C. Standard Plate Incorporation Assay

In no case was there a 2-fold or greater increase in the mean number of revertant testing strains TA98, TA100, TA1537, TA1535 and WP2uvrA in the presence of the test solution compared with the mean of vehicle control value. The positive controls mean exhibited at least a 3-fold increase over the respective mean of the *Salmonella typhimurium* and *Escherichia coli* tester strains used. The results are summarized in Appendix 2.

## VII. Conclusion

All criteria for a valid study were met as described in the protocol. The results of the Bacterial Reverse Mutation Assay indicate that under the conditions of this assay, the test article solution was considered to be Non-Mutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2uvrA. The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 100993, Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicology.

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## Appendix 2:

### Bacterial Mutation Assay Plate Incorporation Assay Results

	Concentration µg per Plate	<b>TA98</b>		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	30	22	26
	1500	31	41	36
	500	70	73	72
	150	56	57	57
	50	53	68	61
	15	26	38	32
	5.0	79	57	68
	1.5	42	48	45
Test Solution w/o S9	5000	39	73	56
	1500	56	60	58
	500	78	82	80
	150	52	79	66
	50	91	80	86
	15	53	86	70
	5.0	78	76	77
	1.5	73	63	68
DI Water w/S9		54	58	56
DI Water w/o S9		56	61	59
2-aminoanthracen w/ S9		301	322	312
2-nitrofluorene w/o S9		210	351	281
Historical Count Positive w/S9		<b>43-1893</b>		
Historical Count Positive w/o S9		<b>39-1871</b>		
Historical Count Negative w/S9		<b>4-69</b>		
Historical Count Negative w/o S9		<b>3-59</b>		

\*CFU = Colony Forming Units

\*Mean = Average of duplicate plates

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	Concentration $\mu\text{g}$ per Plate	<i>TA100</i>		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	13	68	41
	1500	124	148	136
	500	196	204	200
	150	172	112	142
	50	172	124	148
	15	196	140	168
	5.0	148	104	126
	1.5	116	80	98
Test Solution w/o S9	5000	84	24	54
	1500	49	102	76
	500	184	124	154
	150	180	128	154
	50	176	144	160
	15	132	152	142
	5.0	136	196	166
	1.5	116	136	126
DI Water w/S9		120	148	134
DI Water w/o S9		124	68	96
2-aminoanthracen w/ S9		630	540	585
Sodium azide w/o S9		840	1104	972
Historical Count Positive w/S9		<b>224-3206</b>		
Historical Count Positive w/o S9		<b>226-1837</b>		
Historical Count Negative w/S9		<b>55-268</b>		
Historical Count Negative w/o S9		<b>47-250</b>		

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	Concentration µg per Plate	<i>TA1537</i>		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	4	4	4
	1500	1	2	2
	500	5	12	9
	150	11	14	13
	50	6	9	8
	15	6	3	5
	5.0	11	9	10
	1.5	10	8	9
Test Solution w/o S9	5000	3	1	2
	1500	6	7	7
	500	11	7	9
	150	4	11	8
	50	13	14	14
	15	6	10	8
	5.0	9	11	10
	1.5	9	10	10
DI Water w/S9		18	33	26
DI Water w/o S9		20	5	13
2-aminoanthracen w/ S9		150	136	143
2-aminoacridine w/o S9		210	202	206
Historical Count Positive w/S9		<b>13-1934</b>		
Historical Count Positive w/o S9		<b>17-4814</b>		
Historical Count Negative w/S9		<b>0-41</b>		
Historical Count Negative w/o S9		<b>0-29</b>		

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\*Mean = Average of duplicate plates

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	Concentration $\mu\text{g}$ per Plate	<b>TA1535</b>		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	5	6	6
	1500	11	24	18
	500	19	20	20
	150	30	21	26
	50	27	24	26
	15	8	30	19
	5.0	26	26	26
	1.5	19	9	14
Test Solution w/o S9	5000	6	3	5
	1500	8	10	9
	500	27	17	22
	150	15	23	19
	50	27	18	23
	15	21	24	23
	5.0	14	36	25
	1.5	25	19	22
DI Water w/S9		18	21	20
DI Water w/o S9		34	20	27
2-aminoanthracen w/ S9		231	304	268
Sodium azide w/o S9		616	632	624
Historical Count Positive w/S9		<b>22-1216</b>		
Historical Count Positive w/o S9		<b>47-1409</b>		
Historical Count Negative w/S9		<b>1-50</b>		
Historical Count Negative w/o S9		<b>1-45</b>		

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	Concentration $\mu\text{g}$ per Plate	<i>WP2uvrA</i>		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	43	35	39
	1500	22	57	40
	500	53	50	52
	150	42	46	44
	50	53	52	53
	15	37	52	45
	5.0	72	72	72
	1.5	46	58	52
Test Solution w/o S9	5000	52	51	52
	1500	63	50	57
	500	62	57	60
	150	47	34	41
	50	55	45	50
	15	49	52	51
	5.0	56	58	57
	1.5	48	42	45
DI Water w/S9		67	55	61
DI Water w/o S9		49	59	54
2-aminoanthracen w/ S9		274	263	269
Methylmethanesulfonate w/o S9		310	324	317
Historical Count Positive w/S9		<b>44-1118</b>		
Historical Count Positive w/o S9		<b>42-1796</b>		
Historical Count Negative w/S9		<b>8-80</b>		
Historical Count Negative w/o S9		<b>8-84</b>		

\*CFU = Colony Forming Units

\*Mean = Average of duplicate plates

This information is presented in good faith but is not warranted as to accuracy of results. Also, freedom from patent infringement is not implied. This information is offered solely for your investigation, verification, and consideration.