

Assay Report No: 991112-01



Biosafety Assay Center
Animal Technology Institute Taiwan
Assay Report

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Assay Report
Biosafety Assay Center, Animal Technology Institute Taiwan

Assay Report

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Name of Substance Assayed: Product Name : Novel Juice Supplied in 6X Loading Buffer
Cat# : LD001-1000

Number of Substance Assayed: 991112-01

Date of Assay Commenced: 11/01/2010

Date of Assay Completed: 12/24/2010

Assay Results:

4.1 The bacterial characteristic verification results for the standard bacterial strains (TA98, TA100, and TA1535) are illustrated on Table 1. The characteristics of the standard bacterial strains (TA98, TA100, and TA1535) were all verified without any deviation.

Table 1. Characteristic Verification Results for the Standard Bacterial Strains (TA98, TA100, and TA1535)

Bacterial Strains Assayed Assay Criteria	TA98	TA100	TA1535	Result Comparison
Histidine Requirement Test I (Biotin Plate)	—	—	—	Conformed
Histidine Requirement Test II (Biotin/Histidine Plate)	+	+	+	Conformed
<i>Rfa</i> Mutation Test	+	+	+	Conformed
$\Delta uvrB$ Mutation Test	—	—	—	Conformed
R-Factor Test	+	+	—	Conformed
<i>pAQ1</i> Plasmid Test	—	—	—	Conformed
Remark: + : Indication of bacterial growth. The <i>rfa</i> test indicates a positive result. — : Indication of no bacterial growth. The <i>rfa</i> test indicates a negative result.				

4.2 AMES Test – Toxicity Test Results:

4.2.1 Before the reactions occurred for the toxicity test of the AMES Test, the original bacterial
T-04-01E

population for the bacterial strain (TA100) being tested was 6.3×10^8 CFU/mL, which conformed to the assessment standard, 10^{6-9} CFU/mL.

4.2.2 For the results of the experiment group deficient of S9, the concentrations of the testing substance, 991112-01, were 1-fold, 5-fold, 25-fold, and 125-fold diluted solutions. The toxicity was absent in all of them (relative viability rate $\geq 100\%$), thus the stock solution was adopted as the maximum dose for the mutagenicity test of the experiment group lacking S9 for the testing substance, 991112-01.

4.2.3 The S9-containing experiment group's results are as illustrated in Table 3. The stock solution of the testing substance, 991112-01, exhibits toxicity (relative viability rate = 0.89%, which is lower than 20%). The relative viability rates for the 5-fold, 25-fold, and 125-fold diluted solutions of the testing substance, 991112-01, were 60.46%, 97.69%, and 89.77% did not exhibit any toxicity. Therefore, the 5-fold diluted solution would be used as the S9-containing experiment group's maximum dose for the mutagenicity test on the testing substance, 991112-01.

4.3 AMES Test - TA98 Mutagenicity Test Results:

4.3.1 Before the reactions occurred for the mutagenicity test of the AMES Test, the original bacterial population for the bacterial strain (TA98) being tested was 1.9×10^9 CFU/mL, which conformed to the assessment standard, 10^{6-9} CFU/mL.

4.3.2 Mutagenicity Test Results for the Positive Control Group:

4.3.2.1 The results of the positive control group from the S9-deficient experiment group are illustrated in Table 2. The positive control group's mean bacterial population was 69.73 times greater than the negative control group's mean bacterial population; the p value was 0.018 and exhibited significance, which was two folds greater and conformed to the assessment standard of p value < 0.05 . Therefore, the reading of the testing substance's results could be conducted.

4.3.2.2 The results of the positive control group from the S9-containing experiment group are illustrated in Table 3. The positive control group's mean bacterial population was 133.09 times greater than the negative control group's mean bacterial population; the p

value was 0.000 and exhibited significance, which was two folds greater and conformed to the assessment standard of p value <0.05 . Therefore, the reading of the testing substance's results could be conducted.

4.3.3 Mutagenicity Test Results for the Testing Substance:

4.3.3.1 The testing substance's results in the S9-deficient experiment group are as illustrated in Table 2. The mean bacterial populations of the 2-fold, 4-fold, 8-fold, and 16-fold diluted solutions for the testing substance, 991112-01, were 1.02-fold, 1.14-fold, 1.12-fold, and 0.98-fold greater than the negative control group's mean bacterial populations, respectively. Such was less than the 2-fold magnitude and did not exhibit any significance. Therefore, this method was applied to perform the assay and did not detect any mutagenicity in the 2-fold, 4-fold, 8-fold, and 16-fold diluted solutions of the testing substance, 991112-01.

4.3.3.2 The testing substance's results in the S9-containing experiment group are as illustrated in Table 3. The mean bacterial populations of the 5-fold, 10-fold, 20-fold, 40-fold, and 80-fold diluted solutions for the testing substance, 991112-01, were 0.80-fold, 0.73-fold, 0.96-fold, 1.10-fold, and 1.29-fold greater than the negative control group's mean bacterial populations, which was less than the 2-fold magnitude and did not exhibit any significance. Therefore, this method was applied to perform the assay and did not detect any mutagenicity in the 5-fold, 10-fold, 20-fold, 40-fold, and 80-fold diluted solutions of the testing substance, 991112-01.

Table 2. TA98 Mutagenicity Test Results (S9-Deficient Experiment Group) for the Testing Substance 991112-01

Sample No. of Repeat	Negative Control Group (D-PBS)	Positive Control Group (4NOP) [#]	Testing Substance 991112-01 (Dilution Factor)				
			1 X	2 X	4 X	8 X	16 X
Repeat 1	21	1482	36	11	20	22	21
Repeat 2	20	1040	33	23	22	22	21
Repeat 3	15	1452	36	24	23	20	14
Mean Bacterial Population \pm Standard Deviation	19 \pm 3	1325 \pm 247	35 \pm 2	19 \pm 7	22 \pm 2	21 \pm 1	19 \pm 4
Mutagenicity = Testing Substance/Negative Control Group	--	69.73 [*]	1.84 [§]	1.02	1.14	1.12	0.98

Note 1: ^{*} Indication of significance ($p < 0.05$)

Note 2: [#] 4NOP (4-nitro-O-phenylenediamine) as positive control group.

Note 3: [§] The mean bacterial population of the testing substance, 991112-01 (stock solution), was 1.84-fold greater than that for the negative control group, which was < 2 -fold, but the p value was 0.001 and exhibited significance.

Table 3. TA98 Mutagenicity Test Results (S9-Containing Experiment Group) for the Testing Substance 991112-01

Sample No. of Repeat	Negative Control Group (D-PBS)	Positive Control Group (2-AF) [#]	Testing Substance 991112-01 (Dilution Factor)				
			5 X	10 X	20 X	40 X	80 X
Repeat 1	21	4368	30	23	40	37	35

Repeat 2	42	4528	23	24	27	30	61
Repeat 3	35	4280	26	25	28	42	32
Mean Bacterial Population \pm Standard Deviation	33 \pm 11	4392 \pm 126	26 \pm 4	24 \pm 1	32 \pm 7	36 \pm 6	43 \pm 16
Mutagenicity = Testing Substance/Negative Control Group	--	133.09 [*]	0.80	0.73	0.96	1.10	1.29
Note 1: [*] Indication of significance ($p < 0.05$)							
Note 2: [#] 2AF (2-aminofluorene) as the positive control group							

4.4 AMES Test - TA100 Mutagenicity Test Results:

4.4.1 Before the reactions occurred for the mutagenicity test of the AMES Test, the original bacterial population for the bacterial strain (TA100) being tested was 1.3×10^9 CFU/mL, which conformed to the assessment standard, 10^{6-9} CFU/mL.

4.4.2 Mutagenicity Test Results for the Positive Control Group:

4.4.2.1 The positive control group's results from the S9-deficient experiment group are as illustrated in Table 4. The mean bacterial population of the positive control group was 28.99 times greater than the negative control group's mean bacterial population. The p value was 0.000 and exhibited significance, which was two folds greater and conformed to the assessment standard of p value < 0.05 . Therefore, the reading of the testing substance's results could be conducted.

4.4.2.2 The results of the positive control group from the S9-containing experiment group are as illustrated in Table 5. The mean bacterial population of the positive control group was 22.10 times greater than the negative control group's mean bacterial population. The p value was 0.000 and exhibited significance, which was two folds greater and conformed to the assessment standard of p value < 0.05 . Therefore, the reading of the testing

substance's results could be conducted.

4.4.3 Mutagenicity Testing Results for the Testing Substance:

4.4.3.1 The testing substance's results in the S9-deficient experiment group are as

illustrated in Table 4. The mean bacterial populations of the 1-fold, 2-fold, 4-fold, 8-fold, and 16-fold diluted solutions for the testing substance, 991112-01, were 1.05-fold, 1.08-fold, 1.09-fold, 1.02-fold, and 0.97-fold greater than the negative control group's mean bacterial populations, which was less than the 2-fold magnitude and did not exhibit any significance. Therefore, this method was applied to perform the assay and did not detect any mutagenicity in the 1-fold, 2-fold, 4-fold, 8-fold, and 16-fold diluted solutions of the testing substance, 991112-01.

4.4.3.2 The testing substance's results in the S9-containing experiment group are as

illustrated in Table 5. The mean bacterial populations of the 5-fold, 10-fold, 20-fold, 40-fold, and 80-fold diluted solutions for the testing substance, 991112-01, were 1.01-fold, 1.03-fold, 0.96-fold, 1.05-fold, and 1.01-fold greater than the negative control group's mean bacterial populations, which was less than the 2-fold magnitude and did not exhibit any significance. Therefore, this method was applied to perform the assay and did not detect any mutagenicity in the 5-fold, 10-fold, 20-fold, 40-fold, and 80-fold diluted solutions of the testing substance, 991112-01.

Table 4. TA100 Mutagenicity Test (S9-Deficient Experiment Group) Results for the Testing Substance 991112-01

Sample No. of Repeat	Negative Control Group (D-PBS)	Positive Control Group (SA) [#]	Testing Substance 991112-01 (Dilution Factor)				
			1 X	2 X	4 X	8 X	16 X
Repeat 1	110	4184	160	149	168	136	144
Repeat 2	180	4288	151	151	162	145	150
Repeat 3	148	4224	150	175	148	165	128

Mean Bacterial Population \pm Standard Deviation	146 \pm 35	4232 \pm 54	154 \pm 6	158 \pm 14	159 \pm 10	149 \pm 15	141 \pm 11
Mutagenicity = Testing Substance/Negative Control Group	--	28.99 [*]	1.05	1.08	1.09	1.02	0.97
Note 1: [*] Indication of significance ($p < 0.05$)							
Note 2: [#] SA (Sodium azide) as positive control group							

Table 5. TA100 Mutagenicity Test (S9-Containing Experiment Group) Results for the Testing Substance 991112-01

Sample No. of Repeat	Negative Control Group (D-PBS)	Positive Control Group (2-AF) [#]	Testing Substance 991112-01 (Dilution Factor)				
			5 X	10 X	20 X	40 X	80 X
Repeat 1	156	3336	146	160	169	159	158
Repeat 2	156	3268	152	159	145	163	155
Repeat 3	150	3604	170	157	131	162	155
Mean Bacterial	154 \pm 3	3403 \pm 178	156 \pm 12	159 \pm 2	148 \pm 19	161 \pm 2	156 \pm 2
Mutagenicity = Testing Substance/Negative Control Group	--	22.10	1.01	1.03	0.96	1.05	1.01
Note 1: [*] Indication of significance ($p < 0.05$)							
Note 2: [#] 2AF (2-aminofluorene) as positive control group							

4.5 AMES Test - TA1535 Mutagenicity Test Results:

4.5.1 Before the reactions occurred for the mutagenicity test of the AMES Test, the original

bacterial population for the bacterial strain (TA1535) being tested was 3.3×10^9 CFU/mL, which conformed to the assessment standard, 10^{6-9} CFU/mL.

4.5.2 Mutagenicity Test Results for the Positive Control Group:

4.5.2.1 The positive control group's results from the S9-deficient experiment group are as illustrated in Table 6. The mean bacterial population of the positive control group was 36.31 times greater than the negative control group's mean bacterial population. The p value was 0.000 and exhibited significance, which was two folds greater and conformed to the assessment standard of p value <0.05 . Therefore, the reading of the testing substance's results could be conducted.

4.5.2.2 The positive control group's results from the S9-containing experiment group are as illustrated in Table 7. The mean bacterial population of the positive control group was 201.33 times greater than the negative control group's mean bacterial population. The p value was 0.000 and exhibited significance, which was two folds greater and conformed to the assessment standard of p value <0.05 . Therefore, the reading of the testing substance's results could be conducted.

4.5.3 Mutagenicity Test Results for the Testing Substance:

4.5.3.1 The testing substance's results in the S9-deficient experiment group are as illustrated in Table 6. The mean bacterial populations of the 1-fold, 2-fold, 4-fold, 8-fold, and 16-fold diluted solutions for the testing substance, 991112-01, were 0.79-fold, 0.88-fold, 0.93-fold, 0.81-fold, and 1.29-fold greater than the negative control group's mean bacterial populations, which was less than the 2-fold magnitude and did not exhibit any significance. Therefore, this method was applied to perform the assay and did not detect any mutagenicity in the 1-fold, 2-fold, 4-fold, 8-fold, and 16-fold diluted solutions of the testing substance, 991112-01.

4.5.3.2 The testing substance's results in the S9-containing experiment group are as illustrated in Table 7. The mean bacterial populations of the 5-fold, 10-fold, 20-fold, 40-fold, and 80-fold diluted solutions for the testing substance, 991112-01, were 0.88-fold, 1.19-fold, 1.17-fold, 0.83-fold, and 0.76-fold greater than the negative control

group's mean bacterial populations, which was less than the 2-fold magnitude and did not exhibit any significance. Therefore, this method was applied to perform the assay and did not detect any mutagenicity in the 5-fold, 10-fold, 20-fold, 40-fold, and 80-fold diluted solutions of the testing substance, 991112-01.

Table 6. TA1535 Mutagenicity Test (S9-Deficient Experiment Group) Results for the Testing Substance 991112-01

Sample No. of Repeat	Negative Control Group (D-PBS)	Positive Control Group (SA) [#]	Testing Substance 991112-01 (Dilution Factor)				
			1 X	2 X	4 X	8 X	16 X
Repeat 1	11	501	18	10	11	5	17
Repeat 2	14	528	7	13	11	15	18
Repeat 3	17	496	8	14	17	14	19
Mean Bacterial	14±3	508±17	11±6	12±2	13±3	11±6	18±1
Mutagenicity = Testing Substance/Negative Control Group	--	36.31	0.79	0.88	0.93	0.81	1.29
Note 1: * Indication of significance ($p < 0.05$)							
Note 2: # SA (Sodium azide) as positive control group							

Table 7. TA1535 Mutagenicity Test (S9-Containing Experiment Group) Results for the Testing Substance 991112-01

Sample No. of Repeat	Negative Control Group (D-PBS)	Positive Control Group (2-AF) [#]	Testing Substance 991112-01 (Dilution Factor)				
			5 X	10 X	20 X	40 X	80 X
Repeat 1	13	2784	11	11	15	9	8
Repeat 2	14	2720	15	17	16	10	15

Repeat 3	16	2952	11	22	18	16	9
Mean Bacterial	14±2	2819±120	12±2	17±6	16±2	12±4	11±4
Mutagenicity = Testing Substance/Negative Control Group	--	201.33	0.88	1.19	1.17	0.83	0.76

Note 1: * Indication of significance ($p < 0.05$)

Note 2: # 2AA (2-amino-anthracene) as positive control group

Assay Performed by: Yihsing Yao