TEST REPORT

DANAK
TEST Reg. No 145

LAB Scantox

Acyltransferase BL1
(*Bacillus licheniformis* strain BML780-KLM3' CAP50)(GICC 3265)

AMES TEST

LAB Scantox Study No:	62127
Date:	16 October 2006
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Sponsor:	Genencor International Inc. (A Danisco Company) 925 Page Mill Road Palo Alto, CA 94304 USA

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study described in this report "Acyltransferase BL1 (*Bacillus licheniformis* strain BML780-KLM3' CAP50) (GICC 3265) - Ames Test" was conducted under my supervision and responsibility and in compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997), which are in conformity with other international GLP regulations.

This report is a complete and accurate account of the methods employed and the data obtained.

C N Edwards 16 October 2006

C Nicholas Edwards, PhD
Study Director
LAB Scantox

Date

QUALITY ASSURANCE STATEMENT

Study number: 62127

Study title: Acyltransferase BL1 (*Bacillus licheniformis* strain BML780-KLM3+ CAP50) (GICC 3265) - Ames Test

Process-based and facility inspections are carried out to cover the activities within short term studies of the type described in this study report.

A review of the study plan has been performed and reported to the Study Director:

Date of review:	**Reporting date:**
06 March 2006	06 March 2006

In accordance with LAB Scantox Quality Assurance Procedures and relevant parts of current OECD series on principles of Good Laboratory Practice and Compliance Monitoring, the study procedures applicable to this study have been inspected as follows.

Process-based inspection	**Inspection date(s)**	**Reporting date to Study Director and management**
Most recent inspection of similar study	25 January 2006 25 July 2006	25 January 2006 28 July 2006

The study report has been audited. As far as can be reasonably established, the methods, procedures and observations have been accurately described, and the results and data presented in the study report accurately reflect the raw data generated during the study.

The study report gives an accurate account of the methods and procedures outlined in the study plan and in the LAB Scantox Standard Operating Procedures.

Audit date of Draft Report and data:	**Reporting date:**
10 October 2006	10 October 2006
Audit date of Final Report:	Not applicable
16 October 2006	

Pauline Sylvest Salanti
Head of Quality Assurance
LAB Scantox

16 October 2006
Date

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SUMMARY

This study was conducted at LAB Scantox, Hestehavevej 36A, Ejby, DK-4623 Lille Skensved, Denmark.

The test item, Acyltransferase BL1 (*Bacillus licheniformis* strain BML780-KLM3' CAP50) (GICC 3265), was tested in the Ames Test using *Salmonella typhimurium* strains TA 102, TA 100, TA 98, TA 1537, and TA 1535. The test was performed in accordance with the OECD guideline "Bacterial Reverse Mutation Test", No. 471 (1997).

Following a preliminary toxicity test in strain TA 98, Acyltransferase BL1 was tested in two independent main tests. The 'treat and plate' treatment method was used in each test to avoid the possibility that bio-available histidine in the test item might compromise the test. The bacteria were treated with solutions of the test item prepared in sterile saline solution (0.9% NaCl). Seven sequential dose levels selected from within the range 0.16 to 5000 µg/plate were used in the main tests. All the dose levels in this report are expressed in terms of the weight of the freeze-dried sample of the test item as received. Negative control plates were treated by the addition of sterile saline solution (300 µl/plate). The treatments were performed both with and without a metabolic activation system (S-9 mix). Triplicate plates were prepared at each test point.

A dose-related amount of insoluble material was observed on all plates treated with the test item.

Acyltransferase BL1 was generally toxic to the tester strains. The level of toxicity varied somewhat between the tester strains, between treatments in the absence and presence of S-9 mix, and in a few cases between the tests. In general, severe toxicity was observed at the highest one to three dose levels selected for the tests: microcolonies were observed on the plates. Reduced growth of the background lawn was also observed in TA 1537 at three dose levels with S-9 mix in both tests. The test item was not toxic to TA 100 with S-9 mix and TA 98 with S-9 mix in both main tests, but in these cases the highest dose level was 5000 µg/plate, the maximum required by OECD guideline 471 for materials of low toxicity.

No biologically or statistically significant increases in the number of revertant colonies were observed in any tester strain after treatment with Acyltransferase BL1 at any dose level, either in the absence or presence of S-9 mix.

Results obtained with the negative and positive controls demonstrated the sensitivity of the tests and the efficacy of the S-9 mix metabolic activation system.

Based on the results obtained in this study, it is concluded that Acyltransferase BL1 (*Bacillus licheniformis* strain BML780-KLM3' CAP50)(GICC 3265) has not shown any evidence of mutagenic activity.

INTRODUCTION

The Ames Test is a short term *in vitro* mutagenicity test for the evaluation of possible mutagenic effects of chemicals. This test was conducted in accordance with the OECD guideline "Bacterial Reverse Mutation Test", No. 471 (1997).

The experimental work was performed between 11 July 2006 and 14 August 2006. This study was conducted at LAB Scantox, Hestehavevej 36A, Ejby, DK-4623 Lille Skensved, Denmark.

Personnel involved in the study

Study Director: C. Nicholas Edwards, PhD
Sponsor Monitor: Dr Quang Q Bui, Genencor International Inc. (A Danisco Company).

General description of the test system

The ability of the test item to produce mutagenic effects was assessed using the following five bacterial tester strains:

Salmonella typhimurium TA 102
Salmonella typhimurium TA 100
Salmonella typhimurium TA 98
Salmonella typhimurium TA 1537
Salmonella typhimurium TA 1535

Unlike wild-type *Salmonella typhimurium*, these tester strains carry a mutation in the operon (gene) for synthesis of the amino acid histidine. Therefore, the bacteria are not able to grow in substrate without histidine.

The potential mutations are either base-pair substitutions or frameshifts. A base-pair substitution occurs when one base-pair in a DNA sequence is exchanged with a different base-pair, while a frameshift mutation occurs when one or more base-pairs are added to or deleted from a DNA sequence.

When the tester strains are exposed to a test item, mutations may be induced in the defective gene, whereby some of the bacteria can revert to the wild-type. The revertants will be able to grow and form colonies on agar substrate without histidine.

Some chemicals do not exert a mutagenic effect in this system unless they have been changed to active metabolites by mammalian enzymes. The test item was tested both in the presence and absence of a metabolic activation system (S-9 mix). S-9 mix consists of salts, co-factors and an enzyme-rich post-mitochondrial fraction prepared from the livers of rats pre-treated with Aroclor® 1254.

A measure of the mutagenic properties of the test item was obtained by comparing for each strain the number of revertant colonies on treated plates with the number of revertant colonies occurring on negative control plates.

MATERIALS AND METHODS

Test item

Acyltransferase BL1 (*Bacillus licheniformis* strain BML780-KLM3' CAP50)(GICC 3265)
Lot No: 20068010
Description: Lyophilized powder
Intended use: Food additive.

The test item was received from Danisco A/S on 04 April 2006. The Sponsor was responsible for preparation and characterisation of the test item. A certificate of analysis for the test item was supplied by the Sponsor and is reproduced in Appendix 2. The samples of the test item were labelled with the Study No. of this study and stored in a freezer at approximately -18°C in the dark. The certificate of analysis indicates that the test item is stable for at least one year when stored frozen.

Preparation of test item formulations

On the day before each test, the sample of the test item was moved to a refrigerator at approximately 5°C in the dark. On the day of each test, a sub-sample of the test item was taken. The remainder of the test item was returned to storage at approximately -18°C in the dark after use. On some occasions, several sub-samples were taken at the same time and stored in a freezer at approximately -18°C in the dark until use to avoid repeated freeze-thaw cycles of the stock sample. Immediately before use, a sub-sample was dissolved in sterile saline solution (0.9% NaCl, Fresenius Kabi Norge AS) to a range of concentrations so that the required final dose levels were achieved by adding a constant volume of the formulations to the bacteria. The dose levels were expressed in terms of the weight of the test item as received. No analyses were performed to determine the test item concentrations, homogeneity or stability achieved in the formulations or test plates.

Tester strains

The bacterial tester strains listed below were obtained from Professor B. Ames, University of California, Berkeley, USA and the National Collection of Type Cultures, Public Health Laboratory Service, London NW9 5DF, England.

Strain	Target Mutations	Mutation Type	Excision Repair	Cell wall	Plasmid
TA 102	hisG428	Base-pair substitution	-	rfa	pKM101, pAQ1
TA 100	hisG46	Base-pair substitution	uvrB	rfa	pKM101
TA 98	hisD3052	Frameshift	uvrB	rfa	pKM101
TA 1537	hisC3076	Frameshift	uvrB	rfa	
TA 1535	hisG46	Base-pair substitution	uvrB	rfa	

His⁻ mutations

As described in the Introduction, the tester strains carry mutations in the histidine operon. Most of the strains carry a single copy of the mutant gene in the bacterial chromosomal DNA, but strain TA 102 carries the mutation in about 30 copies of the pAQ1 plasmid in each cell. The presence of these mutations was checked by comparing the numbers of revertant colonies on the negative control plates with the historical control data for this laboratory.

uvrB mutation

This mutation affects the operon for the excision repair process and results in a reduced capacity for repair of damage to DNA. The presence of this mutation was checked by testing the sensitivity of the strains to ultraviolet light. The four strains with this mutation were very sensitive to ultraviolet damage as compared to similar wild type strains which were proficient in excision repair. Cross-linking mutagens are most easily detected in strain TA 102 which lacks the uvrB mutation (it has wild-type function).

rfa mutation

This mutation causes partial loss of the lipopolysaccharide barrier of the bacteria, thereby increasing the permeability of the cell wall to large molecules. The presence of the rfa mutation was checked by testing the sensitivity of the strains to the toxic effect of crystal violet.

R-factor plasmid (pKM 101)

Three of the tester strains carry the R-factor plasmid pKM 101, which further increases the sensitivity of these strains by enhancing an error-prone DNA repair process. The plasmid also carries a gene for ampicillin resistance. The presence of the plasmid was checked by testing the resistance of the strains to ampicillin.

R-factor plasmid (pAQ1)

Tester strain TA 102 carries the R-factor plasmid pAQ1. This plasmid carries the hisG428

gene. The plasmid also carries a gene for tetracycline resistance. The presence of the plasmid was checked by testing the resistance of the strain to tetracycline.

Stock cultures

Frozen stock cultures were prepared from the received strains and kept in a liquid nitrogen tank at -196°C, or in a freezer at -80°C. Freshly grown broth cultures were supplemented with 9% DMSO and frozen in a -20°C freezer for one day, then the cryotubes were transferred to a liquid nitrogen tank (-196°C), or a freezer (-80°C). Master agar plates were prepared for frequently used strains and stored at approximately 4°C.

Cultures for mutagenicity testing

Broth cultures for mutagenicity testing were inoculated directly from frozen stock cultures or master plates and incubated on a mixing board at 37°C until a density of approximately 10^9 bacteria/ml was reached (this was checked in each test by plating dilutions of the cultures on nutrient agar). Only freshly grown cultures were used for mutagenicity testing.

Negative and positive controls

Positive and negative (solvent) controls were included in all tests. The negative control treatments show the number of 'spontaneous' revertant colonies. The negative control used in this study was sterile saline solution (0.9% NaCl, 300 µl/plate).

The positive control agents used without S-9 mix were cumene hydroperoxide (100 µg/plate) for TA 102, sodium azide (1 µg/plate) for TA 100 and TA 1535, 2-nitrofluorene (1 µg/plate) for TA 98, and 9-aminoacridine (80 µg/plate) for TA 1537. The positive control agent used with S-9 mix was 2-aminoanthracene (2-AA, 2 µg/plate) for TA 100, TA 98, TA 1537 and TA 1535 and 2-AA (4 µg/plate) for TA 102.

In a separate validity test, the batch of S-9 homogenate used in this study produced large increases in the numbers of revertant colonies of strains TA 98 and TA 100 on plates treated with benzo[a]pyrene at 5 µg/plate.

Rat liver post-mitochondrial fraction

SPF Wistar rats of the stock Mol:WIST were obtained from Taconic Europe, Ejby, DK-4623 Lille Skensved, Denmark. Rats weighing approximately 200 g were used for induction of liver enzymes. A single intraperitoneal injection of Aroclor® 1254 at a dose of 500 mg/kg body weight was given to each rat. The animals were killed with a high concentration of carbon dioxide 5 days after being injected and following a 16-hour period of fasting.

All steps in the preparation of the liver homogenate were performed on ice using aseptic techniques and cold sterile solutions. The livers were removed and minced in 0.15 M KCl

solution (3 ml KCl solution per gram wet liver). After homogenisation the preparation was centrifuged at 9000 g for 15 minutes at approximately 4°C. The supernatant (post-mitochondrial fraction) was decanted, frozen and stored at -196°C until use.

S-9 mix

	Volume	Final concentration
S-9 post-mitochondrial fraction	3.0 ml	5 %
Sodium phosphate buffer (0.2 M, pH 7.4)	30.0 ml	100 mM
Salt solution (0.4 M $MgCl_2$, 1.65 M KCl)	1.2 ml	8 mM/ 33 mM
Glucose-6-phosphate (1.0 M)	0.3 ml	5 mM
NADP (0.1 M)	2.4 ml	4 mM
Distilled water	23.1 ml	

The S-9 mix was prepared in the proportions indicated above shortly before use. The co-factor/buffer mix was sterilized by filtration before addition of the S-9 fraction. The S-9 mix was kept on ice until use.

Media

The media listed below were obtained from Molecular Toxicology Inc., Boone, North Carolina, USA.

Minimal glucose agar plates, containing 1.5% agar and 2% D-glucose in Vogel-Bonner medium E (formulated as described by Maron and Ames (1983)), Moltox™ No. 21-40S21.

Top agar, Moltox™ No. 26-501. This was melted, then cooled to approximately 45°C and supplemented by adding 0.5 mM L-histidine/ D-biotin, Moltox™ No. 26-700 at 10% v/v before use.

Nutrient agar plates (Oxoid No. 2), Moltox™ No. 21-100.

Nutrient broth (Oxoid No. 2), Moltox™ No. 26-505.

TEST PERFORMANCE

A preliminary toxicity test was performed in strain TA 98. Subsequently, two independent main tests were performed using all five strains. Each test included treatments both with and without S-9 mix. Triplicate plates were used at each test point. Positive and negative controls with and without S-9 mix were included in all tests. Parts of the first main test were repeated using a higher range of dose levels because no toxicity was observed at the highest dose level used in the original first main test.

The tests were performed using the 'treat and plate' treatment method (see below) in order to avoid the possibility that bio-available histidine in the test item might cause dose-related increases in the growth of the background lawn of non-revertant bacteria and the numbers of revertant colonies if the plate incorporation or pre-incubation treatment methods had been used. In the treat and plate method, the bacteria were incubated with the test item, nutrient broth and buffer or S-9 mix for a treatment period of 3.5 hours and then the bacteria were washed to remove the test item and any histidine before mixing with top agar and plating on selective agar plates.

Dosing procedures

The bacteria were treated by the addition of aliquots of the test item formulations to the bacteria (300 µl/plate). Five dose levels of the test item were used in the preliminary toxicity test: 50, 160, 500, 1600 and 5000 µg/plate. The highest dose level tested (5000 µg/plate) is the maximum required by the OECD guideline 471 for materials of low toxicity. The lower dose levels were spaced at approximately half-log ($\sqrt{10}$) intervals. The main tests were performed using seven sequential dose levels selected from the following: 0.16, 0.5, 1.6, 5, 16, 50, 160, 500, 1600 and 5000 µg/plate. The seven dose levels selected varied somewhat between tester strains, treatments with and without S-9 mix and in a few cases between the first and second main tests. The selection of the dose levels was made with the expectation that clear signs of toxicity would be observed at the highest dose level. The first main test with strains TA 102 and TA 1535 without S-9 mix was repeated using a higher range of dose levels because no toxicity was observed in the original test. All the dose levels in this report are expressed in terms of the weight of the test item as received.

The bacterial cultures prepared for the test (at a density of approximately 10^9 bacteria/ml) were centrifuged at 1700 g for 10 minutes, the supernatant was discarded and the bacteria were resuspended in one third of the original volume of fresh broth. Test tubes were prepared containing test item formulation (300 µl), vehicle (300 µl), or positive control solution (50 or 100 µl), S-9 mix or 0.02M phosphate buffer pH 7.4 (0.5 ml) and concentrated bacterial suspension (0.1 ml). After incubation at approximately 37°C for 30 minutes with gentle shaking, nutrient broth (0.5 ml) was added to each test tube and the incubation was continued for a further 3 hours. After this incubation period, the bacteria were sedimented by centrifugation, the supernatant was removed and the bacteria were resuspended in buffer (2 ml). After another centrifugation and removal of supernatant, the bacteria were resuspended in buffer (0.5 ml) and top agar (2 ml) was added. The contents of each tube were mixed with a vortex mixer and spread on selective agar plates.

In each test, the plates were incubated at 37°C for approximately 72 hours, and then the number of revertant colonies on each plate was counted.

Toxicity was assessed in all tests by examining the plates for thinning of the background lawn of non-revertant bacteria, the appearance of micro-colonies, or a reduction in the number of

revertant colonies on the test plates in comparison with the negative control plates.

Evaluation of data

The numbers of revertant colonies at each treatment test point were compared to the corresponding negative control values using the Analysis of Variance test. When this test showed statistically significant differences in the data, Dunnett's test was used to determine the statistical significance of increases and decreases in the numbers of revertant colonies for each set of triplicate plates. The statistical analyses were performed with SAS® procedures (version 8.2) described in SAS/STAT® User's Guide, SAS OnlineDoc®, 1999, SAS Institute Inc., Cary, North Carolina 27513, USA.

The tests reported were considered to be valid as all of the following criteria were met:
- negative and positive control data were consistent with the historical control data for this laboratory (see Appendix 1)
- the positive control data showed marked increases over the concurrent negative control values
- the evaluation of the data was not restricted by loss of plates (e.g. through contamination).

The test item would have been considered to have shown evidence of mutagenic activity in this study if all of the following criteria had been met:
- increases in the numbers of revertant colonies were observed at one or more test points
- the mean number of revertant colonies at the test point showing the largest increase was more than twice the corresponding negative control value
- there was a credible scientific explanation for the observed dose-response relationship that involved a mutagenic effect of the test item
- the increases were reproducible between replicate plates and were observed in both main tests
- the increases were statistically significant
- the increases were not directly related to increased growth of the non-revertant bacteria.

The test item would have been considered to have shown no evidence of mutagenic activity if no increases in the mean number of revertant colonies which exceeded twice the negative control value were observed at any test point. The test item would also have been considered to have shown no evidence of mutagenic activity if moderately larger increases had been observed that were not reproducible, not statistically significant, or which were sporadic (without a scientifically valid explanation for the dose-response relationship that involved a mutagenic effect of the test item).

Archives

For a period of 10 years LAB Scantox will be responsible for the archiving of the following material relating to the study:

Study plan, correspondence, test item receipts, all original data and the final report.

At the end of the storage period LAB Scantox will contact the Sponsor for instructions whether the material should be transferred, retained or destroyed.

RESULTS AND DISCUSSION

The results of the preliminary toxicity test are presented in Table 1. A dose-related amount of insoluble material was observed on all plates treated with the test item. The test item showed severe toxicity to the test bacteria at all five dose levels in the absence of S-9 mix and the two highest dose levels with S-9 mix: microcolonies were observed on the plates.

The results of the main tests are presented in Tables 2-6. A dose-related amount of insoluble material was observed on all plates treated with the test item.

Acyltransferase BL1 was generally toxic to the tester strains. The level of toxicity varied somewhat between the tester strains, between treatments in the absence and presence of S-9 mix, and in a few cases between the tests. In general, severe toxicity was observed at the highest one to three dose levels selected for the tests: microcolonies were observed on the plates. Reduced growth of the background lawn was also observed in TA 1537 at the second to fourth dose levels with S-9 mix in both main tests. The parts of the first main test with TA 102 and TA 1535 treated without S-9 mix were repeated using a higher range of dose levels because no toxicity was observed at any dose level in the original test. The test item was not toxic to TA 100 with S-9 mix and TA 98 with S-9 mix in both main tests, but in these cases the highest dose level was 5000 µg/plate, the maximum required by OECD guideline 471 for materials of low toxicity. A statistically significant reduction in the number of revertant colonies was observed in strain TA 1535 after treatment with the test item at 500 µg/plate with S-9 mix in the first main test. This reduction was dose-related and was caused by the test item. A statistically significant reduction in the number of revertant colonies was also observed in strain TA 100 after treatment at 16 µg/plate with S-9 mix in the first main test, but this effect is not considered to have been caused by the test item because it was small and it was not dose-related. Seven dose levels had been used in all parts of the main tests to provide sufficient data for evaluation even though severe toxicity was observed at the higher dose levels in some cases.

No biologically or statistically significant increases in the number of revertant colonies were observed in any tester strain after treatment with Acyltransferase BL1 at any dose level, either in the absence or presence of S-9 mix.

The negative and positive control values were acceptable and generally compatible with the historical control values for January 2004 to November 2005 for this laboratory (see Appendix 1). One negative control value and one positive control value for TA 102 were slightly lower than the historical control ranges. In addition, the values for the positive control treatments of TA 100 and TA 1535 without S-9 mix (sodium azide, 1 µg/plate) were also below the historical control ranges. However, the historical data were mostly obtained in tests performed using the plate incorporation and pre-incubation treatment methods. The positive control treatments produced increases that were clear and more than 2-fold higher than the corresponding mean negative control value in every case and they are all considered to be acceptable. The results obtained with the negative and positive controls demonstrated the sensitivity of the tests and the efficacy of the S-9 mix metabolic activation system.

CONCLUSION

Based on the results obtained in this study, it is concluded that Acyltransferase BL1 (*Bacillus licheniformis* strain BML780-KLM3' CAP50)(GICC 3265) has not shown any evidence of mutagenic activity.

REFERENCES

1. B.N. Ames, McCann, J. and Yamasaki, E. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test: *Mutation Research* **31**, 347-364 (1975).
2. D.M. Maron and Ames B.N. Revised methods for the Salmonella mutagenicity test *Mutation Res.* **113**, 173-215 (1983).
3. OECD Guideline for Testing of Chemicals No 471: Bacterial Reverse Mutation Test. Adopted 21 July 1997.

Preliminary Toxicity Test with Acyltransferase BL1

DOSE	NUMBER OF REVERTANT COLONIES / PLATE PLATE 1	PLATE 2	PLATE 3	MEAN	S.D.	RATIO
STRAIN TA 98, WITHOUT S-9 MIX						
Control	19	20	35	24.7	9.0	.
5000 µg	M	M	M	.	.	.
1600 µg	M	M	M	.	.	.
500 µg	M	M	M	.	.	.
160 µg	M	M	M	.	.	.
50 µg	M	M	M	.	.	.
2-Nitrofluorene 1 µg	180	124	108	137.3	37.8	5.57
STRAIN TA 98, WITH S-9 MIX						
Control	23	23	31	25.7	4.6	.
5000 µg	M	M	M	.	.	.
1600 µg	M	M	M	.	.	.
500 µg	27	27	11	21.7	9.2	0.84
160 µg	14	22	20	18.7	4.2	0.73
50 µg	33	39	37	36.3	3.1	1.42
2-Aminoanthracene 2 µg	2112	2016	1984	2037.3	66.6	79.38

S.D. = Standard deviation
RATIO = Mean number of revertants on treated plates/mean number of revertants on vehicle control plates
No statistical analysis was performed for the preliminary toxicity test
M = Microcolonies, a toxic effect

Ames Test with Acyltransferase BL1

DOSE	NUMBER OF REVERTANT COLONIES / PLATE PLATE 1	PLATE 2	PLATE 3	MEAN	S.D.	RATIO
STRAIN TA 102, WITHOUT S-9 MIX, MAIN TEST 1						
Control	436	468	396	433.3	36.1	.
160 µg	480	492	468	480.0	12.0	1.11
50 µg	424	434	372	410.0	33.3	0.95
16 µg	392	328	368	362.7	32.3	0.84
5 µg	432	400	444	425.3	22.7	0.98
1.6 µg	356	432	408	398.7	38.9	0.92
0.5 µg	300	408	400	369.3	60.2	0.85
0.16 µg	340	420	372	377.3	40.3	0.87
Cumene hydroperoxide 100 µg	1280	1560	1984	1608.0	354.4	3.71
STRAIN TA 102, WITHOUT S-9 MIX, MAIN TEST 1 (REPEAT)						
Control	340	344	292	325.3	28.9	.
1600 µg	M	M	M	.	.	.
500 µg	M	M	M	.	.	.
160 µg	300	368	288	318.7	43.1	0.98
50 µg	352	336	282	323.3	36.7	0.99
16 µg	364	320	328	337.3	23.4	1.04
5 µg	324	326	360	336.7	20.2	1.03
1.6 µg	332	344	364	346.7	16.2	1.07
Cumene hydroperoxide 100 µg	1280	1536	1440	1418.7	129.3	4.36
STRAIN TA 102, WITHOUT S-9 MIX, MAIN TEST 2						
Control	356	328	356	346.7	16.2	.
160 µg	M	M	M	.	.	.
50 µg	388	312	350	350.0	38.0	1.01
16 µg	340	402	357	366.3	32.0	1.06
5 µg	320	384	372	358.7	34.0	1.03
1.6 µg	296	344	228	289.3	58.3	0.83
0.5 µg	300	328	300	309.3	16.2	0.89
0.16 µg	228	312	340	293.3	58.3	0.85
Cumene hydroperoxide 100 µg	1056	800	992	949.3	133.2	2.74

S.D. = Standard deviation
RATIO = Mean number of revertants on treated plates/mean number of revertants on vehicle control plates
* = Statistically significant at 5% level
** = Statistically significant at 1% level
Otherwise, not statistically significant at 5% level (The positive controls were not included in the statistical analysis)
M = Microcolonies, a toxic effect

Ames Test with Acyltransferase BL1

DOSE	NUMBER OF REVERTANT COLONIES / PLATE PLATE 1	PLATE 2	PLATE 3	MEAN	S.D.	RATIO
STRAIN TA 102, WITH S-9 MIX, MAIN TEST 1						
Control	310	336	304	316.7	17.0	.
5000 µg	M	M	M	.	.	.
1600 µg	M	M	M	.	.	.
500 µg	304	312	280	298.7	16.7	0.94
160 µg	364	340	320	341.3	22.0	1.08
50 µg	316	348	322	328.7	17.0	1.04
16 µg	328	368	312	336.0	28.8	1.06
5 µg	318	336	304	319.3	16.0	1.01
2-Aminoanthracene 4 µg	2720	3008	2560	2762.7	227.0	8.72
STRAIN TA 102, WITH S-9 MIX, MAIN TEST 2						
Control	284	338	400	340.7	58.0	.
5000 µg	M	M	M	.	.	.
1600 µg	M	M	M	.	.	.
500 µg	380	372	404	385.3	16.7	1.13
160 µg	364	396	300	353.3	48.9	1.04
50 µg	308	396	282	328.7	59.7	0.96
16 µg	432	352	320	368.0	57.7	1.08
5 µg	360	320	400	360.0	40.0	1.06
2-Aminoanthracene 4 µg	3360	3200	2624	3061.3	387.1	8.99

S.D. = Standard deviation
RATIO = Mean number of revertants on treated plates/mean number of revertants on vehicle control plates
* = Statistically significant at 5% level
** = Statistically significant at 1% level
Otherwise, not statistically significant at 5% level (The positive controls were not included in the statistical analysis)
M = Microcolonies, a toxic effect

Ames Test with Acyltransferase BL1

DOSE	NUMBER OF REVERTANT COLONIES / PLATE PLATE 1	PLATE 2	PLATE 3	MEAN	S.D.	RATIO
STRAIN TA 100, WITHOUT S-9 MIX, MAIN TEST 1						
Control	144	102	120	122.0	21.1	.
160 µg	M	M	M	.	.	.
50 µg	125	108	130	121.0	11.5	0.99
16 µg	136	104	123	121.0	16.1	0.99
5 µg	124	121	92	112.3	17.7	0.92
1.6 µg	132	140	99	123.7	21.7	1.01
0.5 µg	136	106	114	118.7	15.5	0.97
0.16 µg	152	128	118	132.7	17.5	1.09
Sodium azide 1 µg	251	255	272	259.3	11.2	2.13
STRAIN TA 100, WITHOUT S-9 MIX, MAIN TEST 2						
Control	113	115	102	110.0	7.0	.
160 µg	M	M	M	.	.	.
50 µg	148	109	101	119.3	25.1	1.08
16 µg	86	103	124	104.3	19.0	0.95
5 µg	112	105	117	111.3	6.0	1.01
1.6 µg	97	93	96	95.3	2.1	0.87
0.5 µg	107	117	108	110.7	5.5	1.01
0.16 µg	121	111	125	119.0	7.2	1.08
Sodium azide 1 µg	229	224	220	224.3	4.5	2.04

S.D. = Standard deviation
RATIO = Mean number of revertants on treated plates/mean number of revertants on vehicle control plates
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** = Statistically significant at 1% level
Otherwise, not statistically significant at 5% level (The positive controls were not included in the statistical analysis)
M = Microcolonies, a toxic effect

Ames Test with Acyltransferase BL1

DOSE	NUMBER OF REVERTANT COLONIES / PLATE PLATE 1	PLATE 2	PLATE 3	MEAN	S.D.	RATIO
STRAIN TA 100, WITH S-9 MIX, MAIN TEST 1						
Control	140	168	166	158.0	15.6	.
5000 µg	127	144	148	139.7	11.2	0.88
1600 µg	142	138	152	144.0	7.2	0.91
500 µg	151	173	174	166.0	13.0	1.05
160 µg	169	130	139	146.0	20.4	0.92
50 µg	152	180	142	158.0	19.7	1.00
16 µg	125	125	108	119.3*	9.8	0.76
5 µg	120	129	150	133.0	15.4	0.84
2-Aminoanthracene 2 µg	1632	1728	1632	1664.0	55.4	10.53
STRAIN TA 100, WITH S-9 MIX, MAIN TEST 2						
Control	120	104	113	112.3	8.0	.
5000 µg	M	M	M	.	.	.
1600 µg	105	88	104	99.0	9.5	0.88
500 µg	112	109	99	106.7	6.8	0.95
160 µg	115	101	107	107.7	7.0	0.96
50 µg	120	107	105	110.7	8.1	0.99
16 µg	122	110	102	111.3	10.1	0.99
5 µg	107	93	111	103.7	9.5	0.92
2-Aminoanthracene 2 µg	1032	1112	1268	1137.3	120.0	10.12

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Ames Test with Acyltransferase BL1

DOSE	NUMBER OF REVERTANT COLONIES / PLATE PLATE 1	PLATE 2	PLATE 3	MEAN	S.D.	RATIO
STRAIN TA 98, WITHOUT S-9 MIX, MAIN TEST 1						
Control	34	39	28	33.7	5.5	.
160 µg	M	M	M	.	.	.
50 µg	M	M	M	.	.	.
16 µg	28	32	44	34.7	8.3	1.03
5 µg	29	25	25	26.3	2.3	0.78
1.6 µg	34	35	33	34.0	1.0	1.01
0.5 µg	30	31	35	32.0	2.6	0.95
0.16 µg	25	40	29	31.3	7.8	0.93
2-Nitrofluorene 1 µg	172	173	146	163.7	15.3	4.86
STRAIN TA 98, WITHOUT S-9 MIX, MAIN TEST 2						
Control	25	37	44	35.3	9.6	.
160 µg	M	M	M	.	.	.
50 µg	M	M	M	.	.	.
16 µg	48	40	34	40.7	7.0	1.15
5 µg	35	48	42	41.7	6.5	1.18
1.6 µg	56	34	50	46.7	11.4	1.32
0.5 µg	38	34	31	34.3	3.5	0.97
0.16 µg	31	46	31	36.0	8.7	1.02
2-Nitrofluorene 1 µg	209	178	139	175.3	35.1	4.96

S.D. = Standard deviation
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Ames Test with Acyltransferase BL1

DOSE	NUMBER OF REVERTANT COLONIES / PLATE PLATE 1	PLATE 2	PLATE 3	MEAN	S.D.	RATIO
STRAIN TA 98, WITH S-9 MIX, MAIN TEST 1						
Control	30	32	35	32.3	2.5	.
5000 µg	36	29	32	32.3	3.5	1.00
1600 µg	37	41	26	34.7	7.8	1.07
500 µg	27	29	37	31.0	5.3	0.96
160 µg	39	28	34	33.7	5.5	1.04
50 µg	34	34	31	33.0	1.7	1.02
16 µg	28	31	36	31.7	4.0	0.98
5 µg	35	42	45	40.7	5.1	1.26
2-Aminoanthracene 2 µg	872	908	736	838.7	90.7	25.94
STRAIN TA 98, WITH S-9 MIX, MAIN TEST 2						
Control	48	39	43	43.3	4.5	.
5000 µg	38	31	46	38.3	7.5	0.88
1600 µg	25	33	42	33.3	8.5	0.77
500 µg	52	45	42	46.3	5.1	1.07
160 µg	51	39	45	45.0	6.0	1.04
50 µg	36	63	29	42.7	18.0	0.98
16 µg	55	61	50	55.3	5.5	1.28
5 µg	46	43	45	44.7	1.5	1.03
2-Aminoanthracene 2 µg	1252	1160	1232	1214.7	48.4	28.03

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Ames Test with Acyltransferase BL1

DOSE	NUMBER OF REVERTANT COLONIES / PLATE PLATE 1	PLATE 2	PLATE 3	MEAN	S.D.	RATIO
STRAIN TA 1537, WITHOUT S-9 MIX, MAIN TEST 1						
Control	12	20	12	14.7	4.6	.
160 µg	M	M	M	.	.	.
50 µg	M	M	M	.	.	.
16 µg	M	M	M	.	.	.
5 µg	19	12	8	13.0	5.6	0.89
1.6 µg	12	20	20	17.3	4.6	1.18
0.5 µg	17	19	12	16.0	3.6	1.09
0.16 µg	11	14	17	14.0	3.0	0.95
9-Aminoacridine 80 µg	640	652	580	624.0	38.6	42.55
STRAIN TA 1537, WITHOUT S-9 MIX, MAIN TEST 2						
Control	30	29	30	29.7	0.6	.
160 µg	M	M	M	.	.	.
50 µg	M	M	M	.	.	.
16 µg	M	M	M	.	.	.
5 µg	27	24	51	34.0	14.8	1.15
1.6 µg	31	28	50	36.3	11.9	1.22
0.5 µg	46	37	36	39.7	5.5	1.34
0.16 µg	23	31	29	27.7	4.2	0.93
9-Aminoacridine 80 µg	100	100	140	113.3	23.1	3.82

S.D. = Standard deviation
RATIO = Mean number of revertants on treated plates/mean number of revertants on vehicle control plates
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M = Microcolonies, a toxic effect
T = Reduced growth of the background lawn of non-revertant bacteria, a toxic effect.

Ames Test with Acyltransferase BL1

DOSE	NUMBER OF REVERTANT COLONIES / PLATE PLATE 1	PLATE 2	PLATE 3	MEAN	S.D.	RATIO
STRAIN TA 1537, WITH S-9 MIX, MAIN TEST 1						
Control	10	9	12	10.3	1.5	.
5000 µg	M	M	M	.	.	.
1600 µg	13T	8T	14T	11.7	3.2	1.13
500 µg	11T	12T	13T	12.0	1.0	1.16
160 µg	13T	17T	22T	17.3	4.5	1.68
50 µg	10	14	16	13.3	3.1	1.29
16 µg	16	13	13	14.0	1.7	1.35
5 µg	13	11	20	14.7	4.7	1.42
2-Aminoanthracene 2 µg	224	256	276	252.0	26.2	24.39
STRAIN TA 1537, WITH S-9 MIX, MAIN TEST 2						
Control	13	12	10	11.7	1.5	.
5000 µg	M	M	M	.	.	.
1600 µg	12T	7T	9T	9.3	2.5	0.80
500 µg	11T	14T	14T	13.0	1.7	1.11
160 µg	11T	12T	12T	11.7	0.6	1.00
50 µg	15	11	17	14.3	3.1	1.23
16 µg	16	16	10	14.0	3.5	1.20
5 µg	14	16	12	14.0	2.0	1.20
2-Aminoanthracene 2 µg	150	146	160	152.0	7.2	13.03

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Otherwise, not statistically significant at 5% level (The positive controls were not included in the statistical analysis)
M = Microcolonies, a toxic effect
T = Reduced growth of the background lawn of non-revertant bacteria, a toxic effect.

Ames Test with Acyltransferase BL1

DOSE	NUMBER OF REVERTANT COLONIES / PLATE PLATE 1	PLATE 2	PLATE 3	MEAN	S.D.	RATIO
STRAIN TA 1535, WITHOUT S-9 MIX, MAIN TEST 1						
Control	13	8	11	10.7	2.5	.
160 µg	7	11	10	9.3	2.1	0.88
50 µg	15	14	10	13.0	2.6	1.22
16 µg	14	15	16	15.0	1.0	1.41
5 µg	14	14	14	14.0	0.0	1.31
1.6 µg	8	10	12	10.0	2.0	0.94
0.5 µg	9	11	16	12.0	3.6	1.13
0.16 µg	16	14	13	14.3	1.5	1.34
Sodium azide 1 µg	70	67	62	66.3	4.0	6.22
STRAIN TA 1535, WITHOUT S-9 MIX, MAIN TEST 1 (REPEAT)						
Control	16	12	8	12.0	4.0	.
1600 µg	M	M	M	.	.	.
500 µg	M	M	M	.	.	.
160 µg	M	M	M	.	.	.
50 µg	21	22	19	20.7	1.5	1.72
16 µg	14	17	12	14.3	2.5	1.19
5 µg	10	12	20	14.0	5.3	1.17
1.6 µg	21	13	11	15.0	5.3	1.25
Sodium azide 1 µg	71	88	73	77.3	9.3	6.44
STRAIN TA 1535, WITHOUT S-9 MIX, MAIN TEST 2						
Control	22	15	11	16.0	5.6	.
160 µg	M	M	M	.	.	.
50 µg	16	18	13	15.7	2.5	0.98
16 µg	17	12	29	19.3	8.7	1.21
5 µg	10	8	18	12.0	5.3	0.75
1.6 µg	16	15	21	17.3	3.2	1.08
0.5 µg	24	21	22	22.3	1.5	1.40
0.16 µg	15	17	21	17.7	3.1	1.10
Sodium azide 1 µg	64	80	52	65.3	14.0	4.08

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Ames Test with Acyltransferase BL1

DOSE	NUMBER OF REVERTANT COLONIES / PLATE PLATE 1	PLATE 2	PLATE 3	MEAN	S.D.	RATIO
STRAIN TA 1535, WITH S-9 MIX, MAIN TEST 1						
Control	19	14	19	17.3	2.9	.
5000 µg	M	M	M	.	.	.
1600 µg	M	M	M	.	.	.
500 µg	11	6	5	7.3*	3.2	0.42
160 µg	13	15	17	15.0	2.0	0.87
50 µg	20	12	21	17.7	4.9	1.02
16 µg	11	20	21	17.3	5.5	1.00
5 µg	16	19	16	17.0	1.7	0.98
2-Aminoanthracene 2 µg	172	169	195	178.7	14.2	10.31
STRAIN TA 1535, WITH S-9 MIX, MAIN TEST 2						
Control	16	14	19	16.3	2.5	.
5000 µg	M	M	M	.	.	.
1600 µg	M	M	M	.	.	.
500 µg	10	10	10	10.0	0.0	0.61
160 µg	19	19	21	19.7	1.2	1.20
50 µg	19	21	20	20.0	1.0	1.22
16 µg	11	16	18	15.0	3.6	0.92
5 µg	11	21	15	15.7	5.0	0.96
2-Aminoanthracene 2 µg	204	180	224	202.7	22.0	12.41

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M = Microcolonies, a toxic effect

Historical negative and positive control data

January 2004 to November 2005

Strain	Treatment (µg/ml)	S-9 mix	Number of revertant colonies/plate				Number of plates
			Mean	Standard deviation	Minimum	Maximum	
TA 102	Negative control	-	499	126	216	987	189
	Negative control	+	571	160	292	1255	183
	Cumene hydroperoxide (100)	-	2170	615	900	3552	117
	2-Aminoanthracene (4)	+	2833	1193	800	6912	174
TA 100	Negative control	-	134	23	82	218	276
	Negative control	+	144	29	90	286	276
	Sodium azide (1)	-	1343	377	464	2309	264
	2-Aminoanthracene (2)	+	2593	1492	330	7144	264
TA 98	Negative control	-	33	11	12	79	276
	Negative control	+	44	13	20	98	276
	2-Nitrofluorene (1)	-	302	615	87	6003	264
	2-Aminoanthracene (2)	+	1798	1223	158	5692	267
TA 1537	Negative control	-	14	5	5	38	186
	Negative control	+	17	7	3	46	186
	9-Aminoacridine (80)	-	2457	1344	43	6679	221
	2-Aminoanthracene (2)	+	313	591	37	3920	176
TA 1535	Negative control	-	22	8	7	50	183
	Negative control	+	18	7	5	64	183
	Sodium azide (1)	-	948	295	360	1760	174
	2-Aminoanthracene (2)	+	330	242	85	1196	174

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CERTIFICATE OF ANALYSIS

Name of Test Article: **ACYLTRANSFERASE BL1**

Production/Strain Name: *Bacillus licheniformis* **BML780-KLM3' CAP50.**

Production Site: Rochester, USA

Genencor International Culture Collection Number: GICC 3265

Designation of Lot Tested: 20068010

Description: Lyophilized Powder

Analytical studies 3-5 listed below were conducted in accordance with GLP regulations and ISO 9002 standards.

RESULTS:

1. Activity: 21512 LATU/g

2. Dry Matter: 89%

3. Microbial analysis: Microbial analysis conducted on the liquid test material prior to lyophilization by GCOR, Rochester, NY

Analysis	Results
Total viable count	< 1CFU/ml
Coliform	< 1CFU/ml
E. Coli	negative/25 ml
Salmonella	negative/25 ml
Staphylococcus aureus	< 1 CFU/ml
Production strain	negative
Anaerobic sulfite reducers	negative
Antibiotic activity assay	negative

4. Mycotoxin analysis: Not applicable

5. Heavy metals analysis (conducted on the liquid test material prior to lyophilization at Silliker Laboratories)

Analysis	Results
Heavy metals as Pb	< 30 ppm
Arsenic	< 3 ppm
Lead	< 0.5 ppm
Mercury	< 0.5 ppm
Cadmium	< 5 ppm

6. Stability Data : Lyophilized powder is stable for at least 1 year when stored frozen

Bio-Analytical Representative:

Jorn Borch Soe Date: 25. August 2006

Jorn Borch Soe

Study Sponsor's Representative

Hanne Valsted Thyg Date: 25 aug 2006

Hanne Valsted Thygesen

Study Monitor's Representative:

Quang Q. Bui Date: Aug 28, 2006

Quang Q. Bui