TEST REPORT

DANAK
TEST Reg. No 145

LAB Scantox

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

MOUSE MICRONUCLEUS TEST

LAB Scantox Study No:	64145
Date:	13 December 2006
Author:	C. Nicholas Edwards, PhD
Number of pages:	20
Sponsor:	Danisco A/S Edwin Rahrs Vej 38 DK-8220 Brabrand Denmark

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study described in this report "Acyltransferase BL1 (*Bacillus licheniformis* strain BML780-KLM3' CAP50) (GICC 3265) - Mouse Micronucleus Test" was conducted under my supervision and responsibility and is in compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997). These Principles 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 — 13 December 2006

C Nicholas Edwards, PhD
Study Director
LAB Scantox

Date

QUALITY ASSURANCE STATEMENT

Study number: 64145

Study title: Acyltransferase BL1 (*Bacillus licheniformis* strain BML780-KLM3' CAP50) (GICC 3265) - Mouse Micronucleus 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:**
14 September 2006	14 September 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	24 October 2006	24 October 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:**
11 December 2006	11 December 2006
Audit date of Final Report:	Not applicable
13 December 2006	

Pauline Sylvest Salanti
Head of Quality Assurance
LAB Scantox

13 December 2006
Date

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SUMMARY

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

The objective of the study was to determine whether the test item, Acyltransferase BL1 (*Bacillus licheniformis* strain BML780-KLM3' CAP50)(GICC 3265), caused genotoxic effects resulting in the formation of micronuclei in erythrocytes of treated mice. The test item was tested in the Mouse Micronucleus Test performed in accordance with the OECD guideline "Mammalian Erythrocyte Micronucleus Test", No 474 (1997).

In the preliminary toxicity test, groups of two male and two female mice were treated with the test item at dose levels of 1000 and 2000 mg/kg/day by oral gavage on two occasions separated by 24 hours. The dose levels in this report are expressed in terms of the lyophilized test item as received. The highest dose level (2000 mg/kg/day) was the maximum required by the OECD 474 guideline for materials of low toxicity. One female dosed at 2000 mg/kg/day was found dead with blood around its mouth ca. 1.5 hours after the first dose and one male dosed at 1000 mg/kg/day lost 5 g body weight in the 24 hours between the first and second doses, but then remained the same weight in the next 24 hours until sacrifice. None of the other mice showed adverse reactions to treatment. On the basis of these results, the highest dose level selected for the main test was 2000 mg/kg/day. The main test was performed using male mice only since effects observed in the preliminary test did not suggest a substantial difference in toxicity of the test item between the sexes.

In the main test, groups of five male mice were treated with the vehicle (sterile distilled water) or the test item at dose levels of 500, 1000 and 2000 mg/kg/day by oral gavage on two occasions separated by 24 hours. This dose route was selected as it is the principal route of exposure of people in the intended application of the test item. The five positive control group mice were dosed once with Cyclophosphamide at 20 mg/kg by oral gavage. All the mice were killed 24 hours after the final occasion of dosing. Bone marrow smears were prepared on glass slides for each of the mice, stained, and scored using a microscope.

No adverse reactions to treatment were observed in mice treated at any dose level of the test item or in the negative and positive control groups. No evidence that any component of the test item reached the bone marrow was obtained in this study.

No biologically or statistically significant increases in the frequency of micronucleated polychromatic erythrocytes were seen in mice treated with the test item, compared to the negative control values. The positive control treatment caused a large, statistically significant increase, demonstrating the sensitivity of the test system.

It is concluded that Acyltransferase BL1 (*Bacillus licheniformis* strain BML780-KLM3' CAP50)(GICC 3265) did not show any genotoxic activity in this mouse micronucleus test.

INTRODUCTION

The mouse micronucleus test is a short term *in vivo* mutagenicity test for the evaluation of possible genotoxic effects of chemicals. The test was performed in accordance with the OECD guideline "Mammalian Erythrocyte Micronucleus Test", No 474 (1997).

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

The mice for the preliminary toxicity test arrived at LAB Scantox on 13 October 2006 and the slide scoring of the main test was completed on 02 November 2006.

Personnel involved in the study

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

General description of the test system

The micronucleus test, as described by Schmid (1975), is based on the observation that erythroblasts in the bone marrow expel their nucleus in the last stage of erythropoiesis to become polychromatic (immature) erythrocytes (PCE). Acentric chromosome fragments arising from chromosome breakage and also single chromosomes detached from the mitotic spindle will remain in the cell, thereby giving rise to micronuclei which can be observed using a microscope after staining.

A measure of the genotoxic effect of the test item was obtained by comparing the frequency of micronucleated PCE in the bone marrow of treated versus control animals.

Reason for choice of animal species and sex, and route of administration

The mouse was used since it is the most widely used species for this test. The main test was performed using male mice only since effects observed in the preliminary test did not suggest a substantial difference in toxicity of the test item between the sexes. The oral gavage dose route was selected as it is the principal route of exposure of people in the intended application of the test item. The intravenous route, which would have ensured exposure of the target organ to all components of the test item, is not suitable for the dosing of enzyme solutions.

MATERIALS AND METHODS

Test item

Acyltransferase BL1 (*Bacillus licheniformis* strain BML780-KLM3' CAP50)(GICC 03206)

Lot No:	20068010
Description:	Lyophilized powder
Intended use:	Food additive
Stability:	Lyophilized powder is stable for at least 1 year when stored frozen (see Certificate of Analysis in Appendix 2).

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.

Preparation of test item solutions

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 first day of each test, two sub-samples of the test item were taken and the remainder of the test item was returned to storage at approximately -18°C in the dark after use. The first sub-sample was used to prepare the dosing solution(s) for the first day and the second was stored in a freezer at approximately -18°C in the dark until use on the following day. Immediately before use, a sub-sample was dissolved in sterile distilled water (Fresenius Kabi Norge AS). One solution was prepared on each day of dosing for the preliminary toxicity test. The mice were treated with this solution at two dose volumes to achieve the two dose levels. In the main test, the solution prepared at the highest concentration required was diluted with sterile distilled water to a range of concentrations so that the required final dose levels could be achieved by treating the mice with a constant dose volume of the solutions. The dose levels in this report are expressed in terms of the weight of the lyophilized test item as received. No analyses were performed to determine the test item concentrations achieved in the solutions.

Animals

SPF mice of the stock BomTac:NMRI were obtained from Taconic Europe A/S, Ejby, DK-4623 Lille Skensved, Denmark. Four male and four female mice were used in the preliminary toxicity test and twenty eight males were used in the main test. Additional mice were available to replace any that had bodyweights more than 20% from the mean value at the start of dosing. The mice were allowed to acclimatise for at least five days between receipt and use. At the start of dosing the animals were about 7 weeks old and those included in the tests weighed 24 to 29 g for the preliminary toxicity test and 25 to 32 g for the main test. The animals for each test were assigned to the treatment groups using a randomisation scheme.

Housing

The animals were housed in an animal room provided with filtered air at a temperature of 21°C ± 3°C and relative humidity of 55% ± 15%. The room was designed to give 10 air changes per hour. The room was illuminated to give a cycle of 12 hours light and 12 hours darkness. The light was on from 06:00 h to 18:00 h.

The temperature and relative humidity in the animal room were recorded at one minute intervals and the records are retained. The temperature and relative humidity remained within the limits given above throughout this study.

The mice were kept in single-sex groups of two to five in transparent polycarbonate (macrolone type III) cages (floor area: 810 cm^2).

Bedding

The bedding was softwood sawdust from Jelu Werk GmbH, Josef Ehrler GmbH & Co KG, Ludwigsmühle, D-73494 Rosenberg, Germany. Regular analyses for relevant possible contaminants are performed. Certificates of analysis are retained.

Environmental enrichment

The animals were given Aspen Wood Wool from Finn Tapvei Oy, Kortteinen, SF-73600 Kaavi, Finland for environmental enrichment at each change of bedding. An autoclaved brick of aspen wood from the same supplier was also placed in each cage. Regular analyses for relevant possible contaminants are performed. Certificates of analysis for the wood wool and the bricks are retained.

Furthermore, each cage contained a red transparent Mouse House (Noryl, Tecniplast), dimensions: 117 x 117 x 65 mm from Tecniplast Gazzada S.a.r.l., 21020 Buguggiate – Va, Italy. The Mouse House allowed the animals to show a wide range of natural behaviours. Certificates of analysis are retained.

Diet

A complete pelleted rodent diet "Altromin 1314" (for growing animals) supplied by Altromin Gesselschaft für Tierenährung mbH, D-32770 Lage, Germany, was available *ad libitum*. Analyses for major nutritive components and relevant possible contaminants are performed regularly. Certificates of analysis are retained.

Drinking water

The animals had free access to bottles with domestic quality drinking water acidified with hydrochloric acid to pH 2.5 in order to prevent microbial growth. Analyses for relevant possible contaminants are performed regularly. Certificates of analysis are retained.

Animal and cage identification

The cages were identified by cards marked with the study number, group number, and sex of the animals. The animals were identified by unique tail marks.

Treatment

Preliminary toxicity test

Groups of two male and two female mice were dosed with the test item by oral gavage at dose levels of 1000 and 2000 mg/kg body weight/day on two occasions separated by approximately 24 hours. The dose volume was 10 ml/kg body weight for the group dosed at 1000 mg/kg and 20 ml/kg for the group dosed at 2000 mg/kg. The surviving animals were killed approximately 24 hours after the second occasion of dosing. No bone marrow smears were prepared from these mice.

Main test

Groups of five male mice were treated with sterile distilled water (the negative control group) or the test item at 500, 1000 and 2000 mg/kg/day by oral gavage on two occasions separated by approximately 24 hours. In addition, three additional male mice were dosed with the test item at 2000 mg/kg/day as replacements in the event that any of the group of five males dosed at this dose level died before the scheduled sacrifice time. The dose volume for these mice was 20 ml/kg. A positive control group of five male mice was treated with Cyclophosphamide (Baxter Oncology, Germany) at 20 mg/kg by oral gavage on one occasion using a dose volume of 10 ml/kg. All animals were killed approximately 24 hours after the final occasion of dosing.

Clinical observations

The mice were examined for visible signs of ill health or reactions to treatment immediately before each occasion of dosing and at intervals until sacrifice. The mice were weighed immediately before each treatment and immediately before sacrifice.

Bone marrow preparation

The mice from the main test were killed by dislocation of the neck at the scheduled sacrifice time and immediately one femur from each mouse was dissected free. The bone marrow was flushed out of each femur into 2.5 ml of foetal calf serum using a syringe and needle. After vortex mixing, the cell suspension was centrifuged for 10 minutes at 1000 rpm and most of the supernatant was removed. The cells were resuspended in the remainder and smeared on clean glass slides. The slide preparations were fixed in methanol and stained with Giemsa. The slides were dried and coverslips were applied using Pertex mountant, not Dammarxylen mountant as specified in the Study Plan. This deviation from the study plan had no significance for this study.

No slides were prepared and no data are reported for the three additional mice dosed at 2000 mg/kg/day as they were not required to replace any of the group of five mice dosed at this dose level.

Microscopic analysis

Prior to microscopic analysis, one slide from each animal was given a code number by a person who was not involved in the microscopic analysis. The code labels covered all unique identification marks on the slides to ensure that they were scored without bias.

The following counts were made for each animal:
Number of polychromatic erythrocytes (PCE) per 1000 erythrocytes.
Number of PCE with micronuclei in 2000 PCE.
Number of normochromatic erythrocytes (NCE) with micronuclei observed during the scoring of 1000 erythrocytes.

After the analysis of all the slides had been completed, the code was broken and the data was collated.

Criteria for identification of micronucleated erythrocytes

A micronucleus was defined in the following way:

- A bluish mauve strongly coloured uniform circular particle in the cell
- The particle should have a certain size, larger than a point, and it should be located in the same plane as the cell (the cell and the micronucleus should be in focus at the same time)
- During focusing, the particle should stay uniform in colour, light refraction and shape within a relatively large interval
- Cells with two or more micronuclei were counted as single micronucleated cells.

Evaluation of results

The frequencies of micronucleated polychromatic erythrocytes in the test and positive control groups were compared to the frequency values found in the vehicle control group. Statistical analysis was performed using a one way Analysis of Variance based on rank values (Blom's method (2)). The statistical analyses were made 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 test item would have been considered to have shown genotoxic activity in this study if all of the following criteria had been met:
- increases in the frequency of micronucleated polychromatic erythrocytes were observed in treated animals compared to the corresponding negative controls
- the increases were dose-related
- the increases were reproducible between the animals of each group
- the increases were statistically significant.

The historical control range for this laboratory (see Appendix 1) would have also been considered in the evaluation of small increases.

The test item would have been considered to have given a negative response if no reproducible, statistically significant increases were observed.

Archives

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

Study plan, correspondence, test item receipts, all original data, microscope slides 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

Preliminary toxicity test

Initially, a group of two male and two female mice was treated with the test item at 2000 mg/kg/day on two occasions separated by 24 hours and surviving mice were killed 24 hours after the second dose. One female in this group was found dead with blood around its mouth ca. 1.5 hours after the first occasion of dosing. No adverse reactions to treatment or marked changes in body weight were observed in the other three mice in this group. A second group of two male and two female mice was dosed at 1000 mg/kg/day about two hours after the first group. No adverse reactions to treatment were observed in these mice, although one male lost 5 g body weight in the 24 hours between the first and second occasions of dosing and then remained the same weight in the next 24 hours until sacrifice (see Table 1). It is not clear whether the effects observed in the preliminary toxicity test were caused by the test item.

On the basis of these results, the dose levels selected for the main test were 500, 1000 and 2000 mg/kg/day. The highest dose level is the maximum required by the OECD 474 guideline for materials of low toxicity. It was decided to perform the main test using male mice only since effects observed in the preliminary test did not suggest a substantial difference in toxicity of the test item between the sexes. It was decided to treat three additional mice with the test item at 2000 mg/kg/day as replacements in the event that any of the group of five males treated at this dose level died before the scheduled sacrifice time.

Main test

Clinical signs and mortality:

No adverse reactions to treatment were observed in the mice of the negative and positive control groups, or the groups dosed with the test item. All the mice survived until the scheduled sacrifice time. Accordingly, no bone marrow smears were prepared from the three additional mice dosed at 2000 mg/kg/day.

Treatment with the test item or the positive control did not have a clear effect on the body weight of the mice in the period between dosing and sacrifice compared to the negative control mice (see Table 2).

Frequency of PCE and micronucleated PCE

The frequencies of polychromatic erythrocytes (PCE) among total erythrocytes and the frequencies of PCE with micronuclei for individual animals are shown in Table 3. A summary of the data and the statistical analysis are presented in Table 4.

The frequencies of micronucleated PCE for the negative control mice and the positive control mice were within acceptable ranges and compatible with the historical control data for this laboratory (see Appendix 1). The increases in the positive control values over the negative control values were large and statistically significant, demonstrating the sensitivity of the test.

The mean frequencies of PCE among total erythrocytes in the groups treated with the test item were similar to the mean negative control value. A small reduction in the mean value was observed in the positive control group although the range of the values for individual animals overlapped with the negative control group values.

No biologically or statistically significant increases in the frequency of micronucleated PCE were seen in the groups of mice treated with Acyltransferase BL1 (*Bacillus licheniformis* strain BML780-KLM3' CAP50)(GICC 3265) compared to the vehicle control group.

No evidence that any component of the test item reached the bone marrow was obtained in this study. Although the Acyltransferase BL1 enzyme might not have reached the target tissue at significant concentrations, other components found in the test sample including products deriving from enzyme activity likely reached the target tissue.

CONCLUSION

It is concluded that Acyltransferase BL1 (*Bacillus licheniformis* strain BML780-KLM3' CAP50)(GICC 3265) did not show any genotoxic activity in this mouse micronucleus test.

REFERENCES

1. W. Schmid, The micronucleus test, *Mutation Research* **31**, 9-15 (1975).
2. G. Blom, *Statistical Estimates and Transformed Beta Variables:* John Wiley and Sons, Inc., New York (1958).
3. OECD Guideline for the Testing of Chemicals No. 474: Mammalian Erythrocyte Micronucleus Test. Adopted 21 July 1997.

Mouse micronucleus test with Acyltransferase BL1
(*Bacillus licheniformis* strain BML780-KLM3' CAP50)(GICC 3265)

Body weight data
Preliminary toxicity test

Treatment	Animal number	Body weight (g) Day 1	Day 2	Change	Day 3	Change
Group 1	1♂	29	29	0	30	+1
Acyltransferase BL1	2♂	29	28	-1	28	-1
(2000 mg/kg)	3♀	25	24	-1	24	-1
	4♀	27	a		a	
Group 2	5♂	26	26	0	27	+1
Acyltransferase BL1	7♂	27	22	-5	22	-5
(1000 mg/kg)	10♀	26	26	0	26	0
	11♀	24	23	-1	23	-1

Day 1	Body weight measured just before dosing
Day 2	Body weight measured ca. 24 hours after first dose
Day 3	Body weight measured ca. 23 hours after second dose (just before sacrifice)
Change	Body weight change since Day 1 measurement
a	Animal 4♀ was found dead with blood around its mouth ca. 1.5 hours after dosing on Day 1

Mouse micronucleus test with Acyltransferase BL1
(*Bacillus licheniformis* strain BML780-KLM3' CAP50)(GICC 3265)

Body weight data
Main test

Treatment	Animal number	Body weight (g)				
		Day 1	Day 2	Change	Day 3	Change
Group 1	101♂	28	28	0	29	+1
Vehicle control	102♂	28	28	0	28	0
(Sterile distilled water)	103♂	28	28	0	29	+1
	104♂	27	27	0	28	+1
	105♂	28	28	0	28	0
Group 2	106♂	28	29	+1	30	+2
Acyltransferase BL1	107♂	27	26	-1	27	0
(500 mg/kg)	108♂	30	30	0	32	+2
	109♂	30	30	0	30	0
	110♂	29	31	+2	33	+4
Group 3	111♂	30	31	+1	32	+2
Acyltransferase BL1	112♂	26	28	+2	28	+2
(1000 mg/kg)	113♂	26	28	+2	29	+3
	114♂	25	26	+1	26	+1
	115♂	28	30	+2	31	+3
Group 4	116♂	28	29	+1	29	+1
Acyltransferase BL1	117♂	27	27	0	28	+1
(2000 mg/kg)	118♂	27	29	+2	30	+3
	119♂	27	27	0	29	+2
	120♂	27	29	+2	30	+3
Group 5	121♂	27	28	-	27	-1
Positive control	122♂	30	32	-	32	0
Cyclophosphamide	123♂	26	28	-	28	0
(20 mg/kg)	124♂	28	29	-	28	-1
	125♂	26	28	-	28	0

Groups 1 to 4

Day 1	Body weight measured just before dosing
Day 2	Body weight measured ca. 24 hours after first dose
Day 3	Body weight measured ca. 24 hours after second dose (just before sacrifice)
Change	Body weight change since Day 1 measurement

Group 5

Day 1	Body weight measured 24 hours before dosing
Day 2	Body weight measured just before dosing (these mice were dosed just once, on day 2)
Day 3	Body weight measured ca. 24 hours after first dose (just before sacrifice)
Change	Body weight change since Day 2 measurement

Mouse micronucleus test with Acyltransferase BL1
(*Bacillus licheniformis* strain BML780-KLM3' CAP50)(GICC 3265)

Data for individual animals

Treatment	Animal number	MnPCE	MnNCE	%PCE
Group 1	101♂	2	0	42.7
Vehicle control	102♂	1	0	48.1
(Sterile distilled water)	103♂	1	0	44.5
	104♂	0	0	50.1
	105♂	2	0	40.7
Group 2	106♂	1	0	42.9
Acyltransferase BL1	107♂	1	0	49.1
(500 mg/kg)	108♂	2	0	45.4
	109♂	1	1	50.3
	110♂	0	0	43.6
Group 3	111♂	2	1	46.0
Acyltransferase BL1	112♂	2	0	44.3
(1000 mg/kg)	113♂	1	0	47.1
	114♂	1	0	40.9
	115♂	2	0	42.6
Group 4	116♂	2	0	42.0
Acyltransferase BL1	117♂	1	0	47.3
(2000 mg/kg)	118♂	1	0	43.3
	119♂	1	0	39.9
	120♂	1	0	43.7
Group 5	121♂	46	2	35.6
Positive control	122♂	58	2	41.5
Cyclophosphamide	123♂	53	1	35.9
(20 mg/kg)	124♂	56	3	39.2
	125♂	68	2	44.5

MnPCE Number of polychromatic erythrocytes (PCE) with micronuclei (2000 PCE scored)
MnNCE Number of normochromatic erythrocytes (NCE) with micronuclei (1000 erythrocytes scored)
%PCE Frequency of PCE among total erythrocytes (%) (1000 erythrocytes scored)

Mouse micronucleus test with Acyltransferase BL1
(*Bacillus licheniformis* strain BML780-KLM3' CAP50)(GICC 3265)

Summary of results and statistical analysis

Group	Treatment	MnPCE Range	MnPCE Mean	%PCE Mean
1	Vehicle control	0 - 2	1.2	45.2
2	Acyltransferase BL1 (500 mg/kg)	0 - 2	1.0 ns	46.3
3	Acyltransferase BL1 (1000 mg/kg)	1 - 2	1.6 ns	44.2
4	Acyltransferase BL1 (2000 mg/kg)	1 - 2	1.2 ns	43.2
5	Cyclophosphamide (20 mg/kg)	46 - 68	56.2 **	39.3

MnPCE Number of polychromatic erythrocytes (PCE) with micronuclei (2000 PCE scored/animal)
%PCE Frequency of PCE among total erythrocytes (%) (1000 erythrocytes scored/animal)

ns Difference from vehicle control not statistically significant at 5% level
** Statistically significant difference from vehicle control at 1% level

Mouse micronucleus test

Historical Control Data
(Data from preceding ten studies)

	Negative control	Positive control Cyclophosphamide (20 mg/ml)
Number of animals	80	47
Minimum value for MnPCE	0	18
Maximum value for MnPCE	4	86
Mean value for MnPCE	1.5	50.1
Standard deviation for MnPCE	0.8	13.0

MnPCE Number of polychromatic erythrocytes (PCE) with micronuclei (2000 PCE scored/animal)

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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