Enzyme Glucansucrase Detection Report

Protocol No.: DET-PRO-049

Name of the product: 3.75oz Lumineux Oral Essentials Sensitivity Tooth Paste

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Approved by: Nour Abochama Date: 2019-04-02

Nour Abochama
Director QA/QC, American Testing Lab Inc.

Approved by: Kaiil Maddahi DDS Date: 4/2/2019

Kourosh Maddahi DDS
Founder, Oral Essentials Inc.

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1 Objective:

To determine the effect of 3.75oz Lumineux Oral Essentials Sensitivity Tooth Paste on Enzyme Glucansucrase concentrations.

2 Scope:

A 3T3 clone A31 Cell Line from mouse was exposed to a 5000 µg/ml solution of test product for 24 hours. A determination of Glucansucrase concentration was performed using HPLC method.

3 Responsibility:

The persons responsible for the process will perform the and record the information. The responsible person will supervise the study, verify the completion of the records and write the report. Quality Assurance will review and approve the Report.

Name of the Personnel	Designation	Signature
__Nour Abochama_____	__Director QA/QC___	Nour Abochama
__Reem Samman_____	___Chemist_____	Reem Samman

4 Materials, Equipments and documents:

List with Equipment No. and all Standard Operation Procedures (SOPs) for the normal processes under test.

4.1 List of Equipment:

Name of Equipment	Equipment No.
HPLC with DA detector	LAB-2426
Balance	LAB-2233
Pipette	LAB-2422
Incubator	LAB-2223
HPLC Column, 4.6×250 mm, Phenomenex	LAB-0179
`pH-Meter	LAB-2150

Complied By: Reem Samman **Date:** 2019-03-26

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5 Analytical Method

5.1 Standers and Reagents:

5.1.1 Sucrose Reference Standard (or equivalent);
5.1.2 Sodium Acetate, ACS grade (or equivalent);
5.1.3 KH2PO4 S grade (or equivalent);
5.1.4 Acetonitrile HPLC grade (or equivalent)
5.1.5 Purified water USP grade (or equivalent);
5.1.6 Nitromethane HPLC grade (or equivalent);
5.1.7 1-propanol H PLC grade (or equivalent);

5.2 Testing Procedure:

5.2.1 The standard reaction for enzyme assay performed in the mixture containing 50 mM sodium acetate (pH 5.2), 100 mM sucrose, and an appropriately diluted enzyme. The reaction mixture was incubated at 30 °C for 30 min. The resulting fructose was measured by dinitrosalicylic acid (DNS) method (Miller 1959). One unit was defined as the amount of enzyme that catalyzes the release of 1 μmol fructose from sucrose per min at 30 °C.

5.2.2 Glycosylation of l-ascorbic acid to ascorbic acid 2-glucoside
The glycosylation reaction mixture contained 50 mM sodium acetate (pH 5.2), 1 mM MgCl2, 0.1% l-ascorbic acid, 100 mM sucrose, and an appropriately diluted enzyme. Then, the reaction mixture was incubated at 30 °C for 3 h.

5.2.3 The products were analyzed by using thin-layer chromatography (TLC). The samples were spotted onto the silica gel 60 F254 TLC plate (Merck, Germany) and developed with a solvent system consisting of nitromethane/1-propanol/water (2:5:1.5, v/v/v). The plate was then visualized by spraying with 10% H2SO4 in methanol.

5.2.4 The products were then analyzed by high-performance liquid chromatography (HPLC), using a Gemini 5 μm C18 110A column (4.6×250 mm, Phenomenex, USA) with 0.1 M KH2PO4 buffer (pH 2.0) as a mobile phase.

5.3 Chromatographic Conditions:

5.3.1	Injection Volume:	5 µL;
5.3.2	Wavelength:	240 nm;
5.3.3	Mobile Phase:	As per section 5.2.1 of this method;
5.3.4	Flow Rate:	0.7 mL/min;
5.3.5	Expected RT:	5.2 min;
5.3.6	Run time:	15 min.

5.4 Injection Run:

5.4.1 1 inj Mobile Phase BLK;
5.4.2 5 inj working standard STD;
5.4.3 1 inj samples;
5.4.4 1 inj control (working standard) QSTD

5.5 System Suitability:

5.5.1 From the 6 injections of standard, area RSD should not be more than 5.0%.
5.5.2 Recovery between standard control and mean area of standard should be within 95.0%-105.0%.

5.6 Calculations:

5.6.1 Calculate against the mean area of first 5 injection of standard.

5.6.1.1 Recovery standard

$$\textbf{\textit{\% Recovery =}} (\textbf{\textit{Area}}_{qstd}\textbf{\textit{/Mean Area}}_{std})\textbf{\textit{×100}}$$

Where:

$Area_{qstd}$	=	Area of peak of interest in standard or Control.
Mean $area_{std}$	=	Mean area of peak of interest in standard

5.6.1.2 Sample Calculation

$$\%\ \text{of 2-glucoside in Products} = (Area_{spl} \text{Mean Area}_{std}) \times (C_{std}/W_{spl}) \times 100\ mL \times 100$$

Where:

$Area_{spl}$	=	Area of 2-glucoside in Sample preparation
Mean $Area_{std}$	=	Mean Area of 2-glucoside in working standard
C_{std}	=	preparation Concentration of standard in working standard (mg/mL)

W_{spl} = Weight of sample (mg)

Complied By: Reem Samman **Date:** 2019-04-02

Reviewed By: Nour Abochama **Date:** 2019-04-02

6 Protocol

6.1 The test item is prepared y adding 10% (v/v) FCS and 1% antibiotics (v/v) to the artificial Saliva
6.2 Prepare 5000 µg/mL solution of Lumineux Oral Essentials Sensitivity Tooth Paste. Expose the
6.3 test item to the product solution for 24, 36, 48, 60, 72, 84 and 96 hours then prepare as described in section 5.4.

7 Approval:

7.1 Submit the Document to QA for review and approval.

8 Results:

Exposure Time	Blank (µg/ml)	Test Product (µg/ml)	Water (µg/ml)	Ref. Product (µg/ml)
0 hours	0.00	0.840	0.840	0.840
24 hours	0.00	0.755	0.840	0.838
36 hours	0.00	0.583	0.839	0.838
48 hours	0.00	0.340	0.840	0.750
60 hours	0.00	0.099	0.840	0.096
72 hours	0.00	0.089	0.838	0.085
84 hours	0.00	0.089	0.840	0.084
96 hours	0.00	0.089	0.840	0.085

9 Conclusion:

The **Glucansucrase** concentration have been 90% decreased in test item after 72 hours of exposure. The solution at 5000 µg/mL is prepared form Lumineux Oral Essentials Sensitivity Tooth Paste

Approved By: Nour Abochama **Date:** 2019-04-02
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