Lipopolysaccharide Detection Report

Protocol No.: DET-PRO-028

Name of the product: Lumineux Oral Essentials clean and fresh

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Approved by: Nour Abochama Date: 2019-03-01

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

Approved by: Kaill Maddahi DDS Date: 3/1/2019

Kourosh Maddahi DDS
Founder, Oral Essentials Inc.

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1 Objective:

To determine the effect of Lumineux Oral Essentials clean and fresh on Lipopolysaccharide (endotoxins) concentrations.

2 Scope:

A 3T3 clone A31 Cell Line from mouse was exposed to a 5000 µg/ml solution of test product for 69 hours. A determination of Lipopolysaccharide 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_____	ras _____

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 FL detector	LAB-2426
Balance	LAB-2233
Pipette	LAB-2422
Incubator	LAB-2223

Complied By: ras **Date:** 2019-02-28

Reviewed By: Nour Abochama **Date:** 2019-02-28

5 Analytical Method

5.1 Description:

5.1.1 The determination of lipid A was based on the quantitative measurement of β-hydroxymyristic acid and β-hydroxylauric acid by reversed-phase HPLC. β-Hydroxy acids were liberated from ester and amide linkages in endotoxins by acid catalyzed methanolysis. The resulting methyl esters were derivatized with 9-anthracene-carboxyl chloride, 9-fluorene-carboxyl chloride and 4-(1-pyrenyl)butyric acid chloride and quantified with a fluorescence detector.

5.2 Injection Run:

5.2.1 1 inj Mobile Phase BLK;
5.2.2 5 inj working standard STD;
5.2.3 1 inj samples;
5.2.4 1 inj control (working standard) QSTD

5.3 System Suitability:

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

5.4 Calculations:

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

5.4.1.1 Recovery standard

$$\%\ Recovery = (Area_{qstd} / Mean\ Area_{std}) \times 100$$

Where:

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

5.4.1.2 Sample Calculation

% of Lipopolysaccharide in Products=(AreasplMean Areastd)×(Cstd/Wspl)×100 mL×100
Where:

$Area_{spl}$	=	Area of Lipopolysaccharide in Sample preparation
Mean $Area_{std}$	=	Mean Area of Lipopolysaccharide in working standard preparation
C_{std}	=	Concentration of standard in working standard (mg/mL)
W_{spl}	=	Weight of sample (mg)

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6 Protocol

6.1 The test item is prepared y adding 10% (v/v) FCS and 1% antibiotics (v/v) to the artificial Sylvia
6.2 Prepare 5000 µg/mL solution of Lumineux Oral Essentials clean and fresh. Expose the test
6.3 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	0.2	0.00	0.2
24 hours	0.00	0.19	0.00	0.2
36 hours	0.00	0.19	0.00	0.2
48 hours	0.00	0.18	0.00	0.19
60 hours	0.00	0.17	0.00	0.17
72 hours	0.00	0.12	0.00	0.13
84 hours	0.00	0.12	0.00	0.13
96 hours	0.00	0.12	0.00	0.13

9 Conclusion:

The Lipopolysaccharide (Endotoxines) concentration have been 48%% decreased in test item after 72 hours of exposure.
The solution at 5000 µg/mL is prepared form Lumineux Oral Essentials clean and fresh Lot# 17K13B2

Approved By: Nour Abochama **Date:** 2019-03-01

Nour Abochama, M. Sc.,