

Mycobacterium smegmatis*, Purified Lipoarabinomannan (LAM)*Catalog No. NR-14849**

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Product Description: NR-14849 is a preparation of lipoarabinomannan (LAM) derived from the cell wall of *Mycobacterium smegmatis*. LAM possesses many biological activities including immunogenicity, induction of TNF and the release of other cytokines, and inhibition of antigen processing. The culture was grown to late-log phase in glycerol-alanine-salts medium, washed with PBS and inactivated by gamma irradiation. The cells were delipidated and suspended in PBS buffer containing 4% Triton X-114 and broken by French Press. The lysate was incubated at 4°C for 18 hours with rocking and insoluble material was removed by centrifugation. The Triton X-114 extract was collected, heated at 37°C to allow biphasic partitioning, and centrifuged. The detergent layer was collected and macromolecules, including LAM, were recovered by ethanol precipitation. The ethanol-insoluble material was suspended in PBS, and the proteins digested and dialyzed out. The crude carbohydrate mixture was fractionated by size exclusion chromatography and the pure LAM pooled. Buffer contaminants were removed by extensive dialysis. Contaminating LPS is avoided as all buffers and water used are endotoxin-free.

Lot: 62575033**Manufacturing Date: 28OCT2015**

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 15.Smeg.10.28.DMH.LAM is attached.

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WORK SHEET FOR LIPOARABINOMANNAN

General Information

BEI Catalog Number: NR-14849
Lot Number: 15.Smeg.10.28.DMHLAM
Species: *Mycobacterium smegmatis*
Strain: N/A

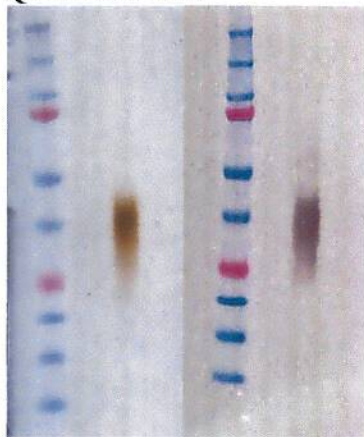
Purification Information

Starting material (lot #): 15.SMEG.6.19.5.Wcg and 15.SMEG.2.20.4.Wcg
Cells Irradiated: Yes
Viability Test Performed: N/A
Cell amount (wet weight): 93.9g
Protocol used (SOP #): PP015.3, PP016.4, PP017.2,
Date started: 07/07/15
Date completed: 10/28/2015
Notebook; pages: DMH 3 p. 1-23
Additional notes (if applicable): N/A

Quality Control Information

NMR results: pass Notebook and page(s): DMH 3 p. 15
* A trace amount of **Tris**(hydroxymethyl)aminomethane was found in the sample NMR.
Endotoxin amount: >0.1 ng/mg Notebook and page(s): DMH 3 p. 20
GC Results: 9.154 mg/ml Notebook and page(s): DMH 3 p. 21
Total amount of LAM: 93.55 mg
Silver stain date: 10/27/15 Notebook and page(s): DMH 3 p. 22
Western blot date: 10/28/15 Antibody used: CS35 Notebook and page(s): DMH 3 p. 22

QC Gel and Blot:



Aliquot Information:

187 vials @ 500 µg
93550 µg total

Dawn DM AF 10/28/15
(Research Associate) (date)

C. McHaffey 10/28/15
(Laboratory Supervisor) (date)