

Mycobacterium tuberculosis*, Strain H37Rv, Purified Lipomannan (LM)*Catalog No. NR-14850**

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Product Description: NR-14850 is a preparation of the lipomannan (LM) derived from the cell wall of irradiated *Mycobacterium tuberculosis*, strain H37Rv. 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, then suspended in a PBS buffer containing 4% Triton X-114 and broken by French Press. This lysate was rocked at 4°C for 18 hours and insoluble material was removed by repeated 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 LM, were recovered by ethanol precipitation. The ethanol insoluble material was suspended in PBS, and the proteins were digested and dialyzed out. The crude carbohydrate mixture was fractionated by size exclusion chromatography and the pure LM pooled. Buffer contaminants were removed by extensive dialysis. Contaminating LPS was avoided as all buffers and water used were endotoxin-free.

Lot: 62976462**Manufacturing Date: 31MAR2016**

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 16.Rv.4.01.DMH.LM.a is attached.

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WORK SHEET FOR LM

General Information

BEI Catalog Number: NR-14850
CSU Lot Number: 16.Rv.4.01.DMH. LM.a
Species: *Mycobacterium tuberculosis*
Strain: H37Rv

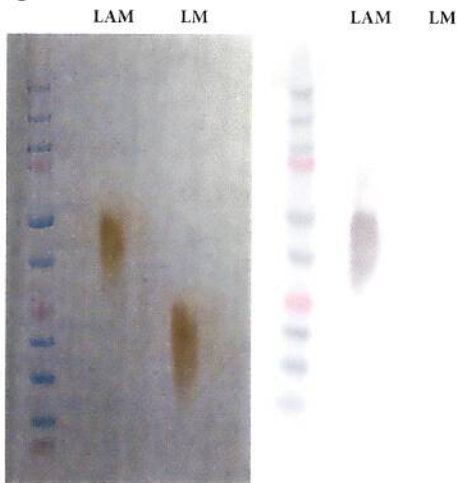
Purification Information

Starting material (lot #): 14.Rv.2.6.10.4.WCg.a
Cells Irradiated: Yes
Viability Test Performed: No Viable Organism Detected
Cell amount (wet weight): 99.5 g
Protocol used (SOP #): PP015.3, PP016.5, PP017.2
Date started: 10/30/15
Date completed: 03/31/16
Notebook; pages: DMH 3 p.25-93, DMH 4 p.1-5
Additional notes (if applicable): N/A

Quality Control Information

NMR results: <u>pass</u>	Notebook and page(s): <u>DMH 3 p.82</u>
Endotoxin amount: <u>3.1 ng/mg</u>	Notebook and page(s): <u>DMH 4 p.3</u>
GC Results: <u>2.17 mg/ml</u>	Notebook and page(s): <u>DMH 4 p.2</u>
Total amount of LM: <u>7.6 mg</u>	
Silver stain date: <u>03/31/16</u>	Notebook and page(s): <u>DMH 4 p.3</u>
Western blot date: <u>03/31/16</u>	Antibody used: <u>CS35</u> Notebook and page(s): <u>DMH 4 p.3</u>

QC Gel:



Aliquot Information:

56 vials @ 100 ug
2 mg in 2 vials unaliquoted
7600 ug total


(Research Associate) (date)


(Laboratory Supervisor) 4-4-16
(date)