

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

This reagent is the tangible property of the U.S. Government.

Product Description: NR-14850 is a preparation of the lipomannan (LM) derived from the cell wall of *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: 62795343**Manufacturing Date: 26AUG2014**

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 14.Rv.8.26.DMH.LM.a is attached.

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WORK SHEET FOR LIPOMANNAN

General Information

Lot Number: 14.Rv.8.26.DMH.LM.a
Species: *Mycobacterium tuberculosis*
Strain: H37Rv

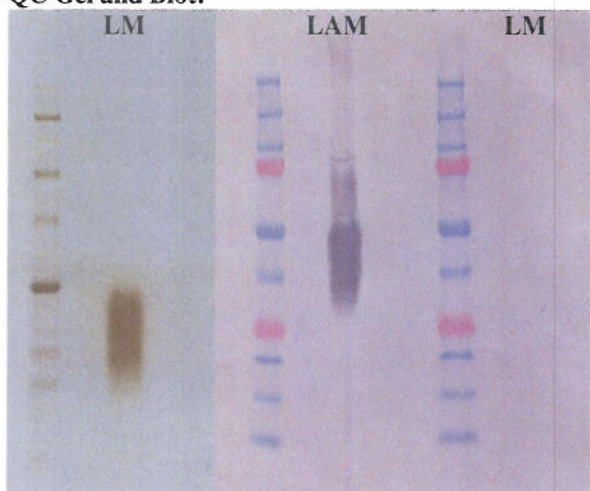
Purification Information

Starting material (lot #): 13.Rv.2.5.28.7.WCg
Cell amount (wet weight): 87.2g
Protocol used (SOP #): PP015.3, PP016.4, PP017.2
Date started: 05/02/14
Date completed: 08/26/2014
Notebook; pages: DMH 1 p. 3-46
Additional notes (if applicable): A small amount of glacial acetic acid was added to half of the HPLC to help resolubilize the pellet.

Quality Control Information

Clarity of product: clear
NMR results: pass Notebook and page(s): DMH 1 p. 41
Endotoxin amount: 1.509 ng/mg Notebook and page(s): DMH 1 p. 42
GC Results: 3.16 mg/ml Notebook and page(s): DMH 1 p. 37
Total volume: 2.3 ml Total amount of LM: 7.268 mg
Silver stain date: 08/13/14 Notebook and page(s): DMH 1 p. 43
Western blot date: 08/13/14 Antibody used: CS35 Notebook and page(s): DMH 1 p. 43

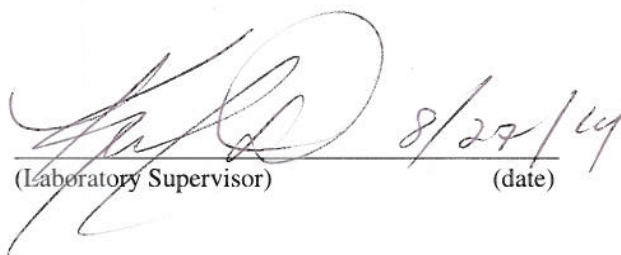
QC Gel and Blot:



Aliquot Information:

72 vials @ 100 μ g
1 vial @ 68 μ g
7268 μ g total


(Research Associate) (date)


(Laboratory Supervisor) (date)