

***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: 61977661****Manufacturing Date: 26JUN2014**

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 14.Rv.6.26.DMH.LM is attached.

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You are authorized to use this product for research use only. It is not intended for human use.



## WORK SHEET FOR LIPOMANNAN

### General Information

Lot Number: 14.Rv.6.26.DMH.LM  
Species: *Mycobacterium tuberculosis*  
Strain: H37Rv

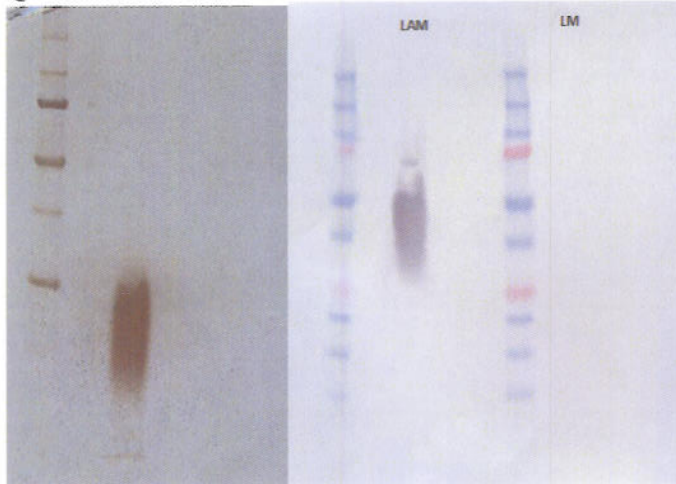
### Purification Information

Starting material (lot #): 13.Rv.2.5.14.6.WCg  
Cell amount (wet weight): 93.4g  
Protocol used (SOP #): PP015.3, PP016.4, PP017.2,  
Date started: 02/21/14  
Date completed: 06/26/2014  
Notebook; pages: SOS/TH 4, p. 77-104. DMH 1 p. 1-28  
Additional notes (if applicable): Passed through a Mustang (Pall) filter to reduce endotoxin.

### Quality Control Information

Clarity of product: <u>clear</u>	Notebook and page(s): <u>DMH 1 p. 8</u>
NMR results: <u>pass</u>	Notebook and page(s): <u>DMH 1 p. 25</u>
Endotoxin amount: <u>3.29 ng/mg</u>	Notebook and page(s): <u>DMH 1 p. 26-27</u>
GC Results: <u>0.515 mg/ml</u>	Total amount of LM: <u>1.57 mg</u>
Total volume: <u>3.05 ml</u>	Notebook and page(s): <u>DMH 1 p. 28</u>
Silver stain date: <u>06/24/14</u>	Antibody used: <u>CS35</u>
Western blot date: <u>05/20/14</u>	Notebook and page(s): <u>DMH 1 p. 11</u>

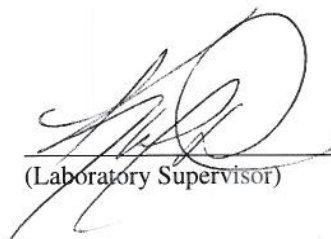
### QC Gel and Blot:



### Aliquot Information:

15 vials @ 100  $\mu$ g  
1 vial @ 70  $\mu$ g  
1570  $\mu$ g total

  
(Research Associate) (date)

  
(Laboratory Supervisor) 6/26/14  
(date)