

***Mycobacterium tuberculosis*, Strain H37Rv, Purified Lipoarabinomannan (LAM)****Catalog No. NR-14848**

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

**Product Description:** NR-14848 is a preparation of the lipoarabinomannan (LAM) derived from the cell wall of irradiated *Mycobacterium tuberculosis*, strain H37Rv. LAM possesses many biological activities including immunogenicity, induction of TNF and the release of other cytokines, and inhibition of antigen processing. The nonreducing termini of H37Rv LAM are extensively capped with mannose. The culture was grown to late-log phase (day 14) in glycerol-alanine-salts (GAS) medium, washed with PBS pH 7.4, 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 at 27,000 x g. The Triton X-114 extract was collected, heated at 37°C to allow biphasic partitioning, and centrifuged at 27,000 x g. 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. The solution was lyophilized and stored dry at -80°C. QC includes SDS-PAGE, Western blotting, LAL assay, NMR, and neutral sugar GC analysis. Contaminating LPS is avoided as all buffers and water used are endotoxin-free.

**Lot: 62676097****Manufacturing Date: 26AUG2014**

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

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## WORK SHEET FOR LIPOARABINOMANNAN

### General Information

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

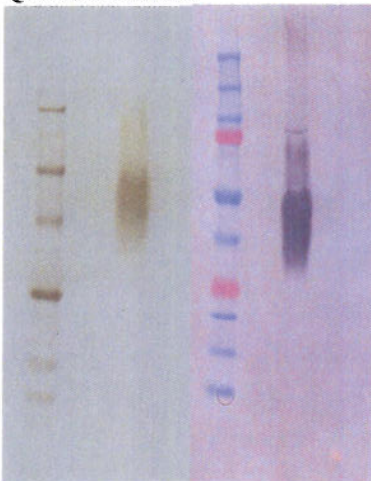
### Purification Information

Starting material (lot #): 13.Rv.2.5.28.7.WCg  
Cell amount (wet weight): 87.2 g  
Protocol used (SOP #): PP015.3, PP016.4, PP017.2  
Date started: 05/02/14  
Date completed: 08/26/2014  
Notebook; pages: DMH 1 p. 3-45  
Additional notes (if applicable): A small amount of glacial acetic acid was added to half of the HPLC to help re-solubilize the pellet.

### Quality Control Information

Clarity of product: slightly cloudy  
NMR results: pass Notebook and page(s): DMH 1 p. 41  
Endotoxin amount: 1.608 ng/mg Notebook and page(s): DMH 1 p. 42  
GC Results: 5.016 mg/ml Notebook and page(s): DMH 1 p. 37  
Total volume: 3.19 ml Total amount of LAM: 16.00 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:**  
32 vials @ 500 µg

16000 µg total

(Research Associate)

(date)

(Laboratory Supervisor)

(date)