

***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 *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: 61983887****Manufacturing Date: 24JAN2014**

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 14.Rv.1.24.TH.ManLAM is attached.

ATCC<sup>®</sup>, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected by the contractor to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC<sup>®</sup>'s knowledge.

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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 PURIFIED LIPOARABINOMANNAN

### General Information

Lot Number: 14.Rv.1.24.TH.ManLAM  
Species: *Mycobacterium tuberculosis*  
Strain: H37Rv

### Purification Information

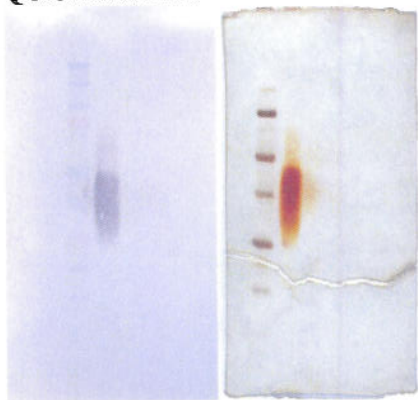
Starting material (lot #): 13.Rv.2.3.12.8.WCg  
Cell amount (wet weight): 90.0g  
Protocol used (SOP #): PP015.3, PP016.4, PP017.2  
Date started: 11 / 15 / 2013  
Date completed: 1 / 24 / 2014  
Notebook; pages Tamara Hess; SOS #4 Rv, pgs. 53-70  
Additional notes (if applicable):

### Quality Control Information

Clarity of product: Clear  
NMR results: Pass  
Endotoxin amount: <0.14 ng ET / mg LAM  
GC/MS Results: 0.794mg/ml  
Total volume: 19.873 ml  
Silver stain date: 1/23/14  
Western blot date: 1/24/14 Antibody used: CS35

Notebook and page(s): SOS #4 Rv, pg. 66  
Notebook and page(s): SOS #4 Rv, pgs. 69  
Notebook and page(s): SOS #4 Rv, pgs. 65-68  
Total amount of LAM: 15.780 mg  
Notebook and page(s): SOS #4 Rv, pg 70  
Notebook and page(s): SOS #4 Rv, pg. 70

### QC Gel and Blot:

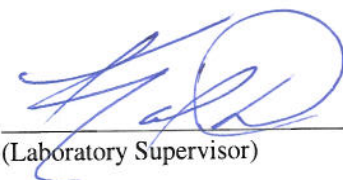


### Aliquot Information:

31 vials @ 500 ug  
1 vial @ 280 ug  
15780 ug total

  
(Research Associate)

 2/11/14  
(date)

  
(Laboratory Supervisor)

2 / 11 / 14  
(date)