

***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: 61181934****Manufacturing Date: 25OCT2012**

QC testing was performed by Colorado State University under the TB Vaccine Testing and Research Materials Contract (NIH). The Colorado State University documentation for lot 12.Rv.2.7.2.SOS.LM 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.

ATCC<sup>®</sup> is a trademark of the American Type Culture Collection.

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



## WORK SHEET FOR PURIFIED LIPOMANNAN

### General Information

Lot Number: 12.Rv.2.7.2.SOS.LM  
Species: *M. tuberculosis*  
Strain: H37Rv

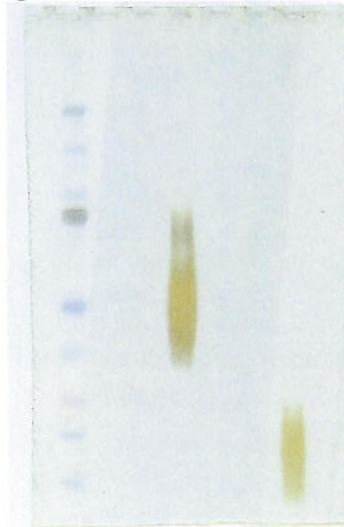
### Purification Information

Starting material (lot #): 11.Rv.2.9.12.6.WCg  
Cell amount (wet weight): 95.7g  
Protocol used (SOP #): PP015.1, PP016.3, PP017.2  
Date started: 07 / 02 / 2012  
Date completed: 10 / 25 / 2012  
Notebook; pages: Shane Orlin Severs; SOS-Mixed, pgs. 22-35  
Additional notes (if applicable):

### Quality Control Information

|                                       |   |
|---------------------------------------|---|
| Clarity of product: Clear             |   |
| NMR results: Pass                     | Notebook and page(s): SOS-Mixed, pg. 32     |
| Endotoxin amount: 0.406 ng ET / mg LM | Notebook and page(s): SOS-Mixed, pg. 35     |
| GC Results: 0.95 mg/ml                | Notebook and page(s): SOS-Mixed, pgs. 33-34 |
| Total volume: 17.15 ml                | Total amount of LM: 16.3 mg                 |
| Silver stain date: 10 / 24 / 2012     | Notebook and page(s): SOS-Mixed, pg. 34     |

### QC Gel:

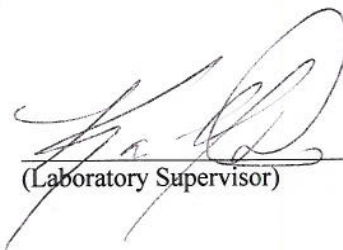


Marker      LAM      LM

**Aliquot Information:**  
163 vials @ 100 ug each

  
(Research Associate)

10-25-2012  
(date)

  
(Laboratory Supervisor)

10/26/2012  
(date)