Certificate of Analysis for NR-56329

Mycobacterium tuberculosis, Strain H37Rv, Purified Demannosylated Lipoarabinomannan (DLAM)

Catalog No. NR-56329
This reagent is the tangible property of the U.S. Government.

Product Description:
NR-56329 is a preparation of demannosylated lipoarabinomannan (DLAM) derived from the cell wall of irradiated *Mycobacterium tuberculosis (M. 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 strain H37Rv LAM are extensively capped with mannose. Mannose-capped LAM (ManLAM) has demonstrated immunomodulatory effects, such as inhibition of T cell activation and proliferation and influences cytokine production. Variability in mannose capping observed in clinical isolates and among different strains of *M. tuberculosis* may contribute to the variation of biological activities *in vitro*. Removal of the mannose caps of LAM from virulent strain H37Rv provides the opportunity to study the biological features attributed to LAM that are not associated with mannose capping.

Lot: 70049264 **Manufacturing Date: 10OCT2022**

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 22.Rv.10.10.DLAM is attached.

ATCC®, 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®'s knowledge.

Support Provided by NIAID

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WORK SHEET FOR DEMANNOSYLATED LIPOARABINOMANNAN

General Information

BEI Catalog Number: NR- 56329
CSU Lot Number: 22.RV.10.10.DLAM
Species: *Mycobacterium tuberculosis*
Strain: H37RV

Purification Information:

Cells Irradiated: Yes
Viability Test performed: No Viable Organisms Detected
*LAM starting material Lot Number: 20.RV.06.10.LAM
LAM starting Material (mgs): 6.5 mgs
Protocols Used (SOP #): PP059 and SP079
Date Started: 08/03/22
Date Completed: 10/10/22
Notebook pages: Megan Stookey NB #1 pgs 54-90
Additional notes: *Will not be found in BEI inventory; product was left over from a different project. QC for starting material also included.

Quality Control Information:

BCA: <1mg/10mgs LAM — Notebook and pages: Megan Stookey NB #1; pgs 59-60
Endotoxin Assay Used: Endozyme II
Endotoxin amount: 2.25ng/mg — Notebook and pages: Megan Stookey NB #1; pg 74
Image J concentration: 0.7191gs/ml — Notebook and pages: Megan Stookey NB #1; pgs 66-69
Total amount of demannosylated LAM: 4.437mgs
Silver Stain: 10/05/22 — Notebook and pages: Megan Stookey NB #1; pg 90
Western Blot: 08/19/22 antibody: Con A — Notebook and pages: Megan Stookey NB #1; pgs 69-72
Western Blot: 08/18/2022 antibody: CS-35 — Notebook and pages: Megan Stookey NB #1; pgs 69-72

Western Blots:

- Lane 1: Ladder
- Lane 2: Demannosylated LAM - 2µg
- Lane 2: H37RV LAM (positive control) - 2µg
- Lane 3: HSPX (recombinant protein; negative control) - 5µg

Silver Stain:

- Lane 1: Ladder
- Lane 3: Demannosylated LAM -5µg
- Lane 5: H37RV LAM (positive control) - 5µg
- Lane 7: HSPX (recombinant protein; negative control) - 5µg

QC Gel and Blots:

Con A WB

CS-35 WB

Silver Stain

Aliquot Information:

17 X 0.25mg BEI labeled aliquots
1 X 0.187mg aliquot in bulk

Research Associate 10/19/22 Date

Laboratory Scientist 10/18/2022 Date

Demannosylated Lipoarabinomannan

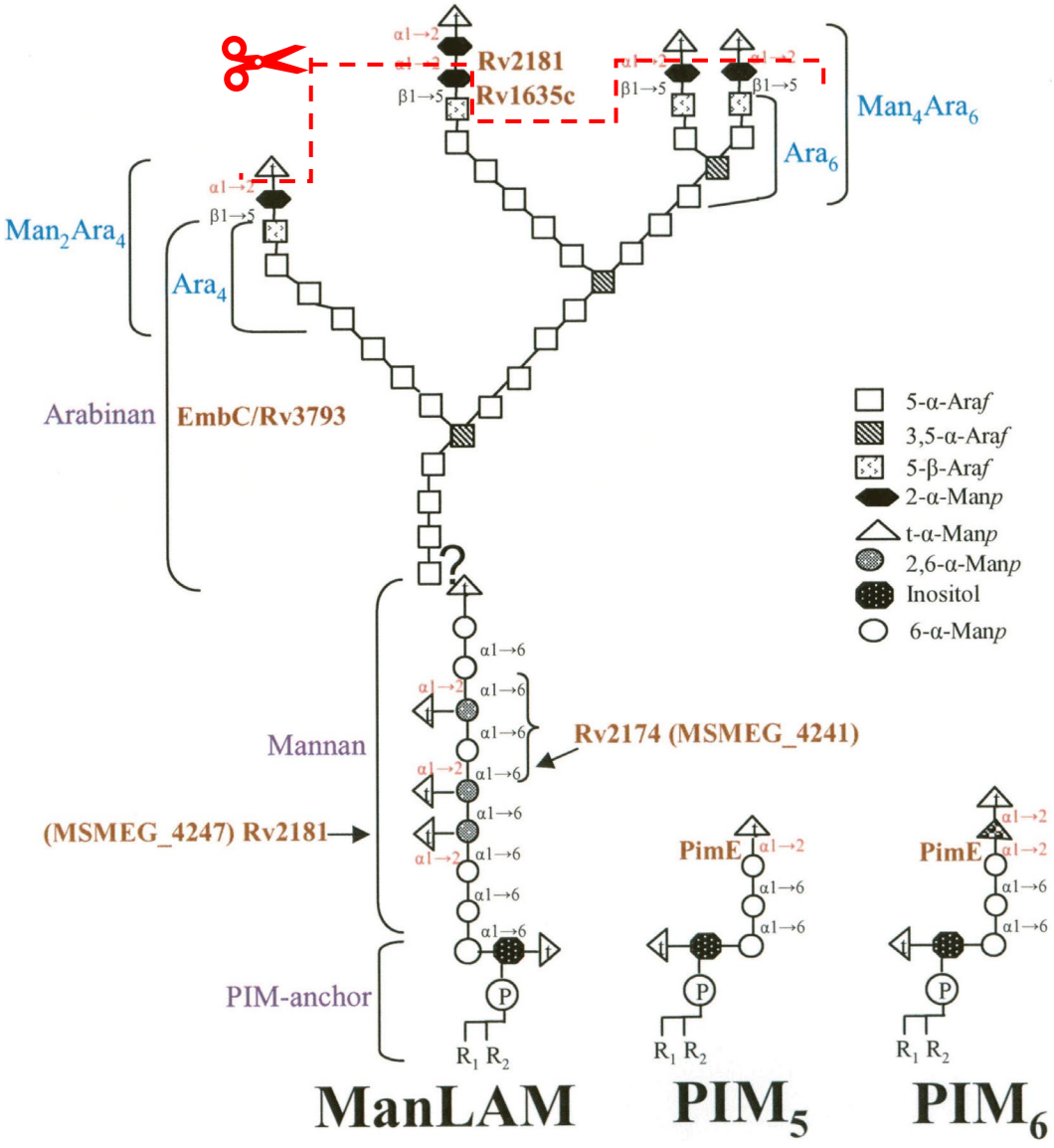

Fig. S1. Schematic structure of ManLAM, PIM_5 and PIM_6. A representation of the structure of ManLAM shows the mannose-capped nonreducing termini, the mannan core, and the phosphatidylinositolmannoside anchor. Although LAM could have a few arabinan chains (not confirmed with data), only 1 chain is shown for simplicity, and not all arabinan termini are capped. The mannan core is characterized by an α(1→6)-linked mannan, substituted at C2 by α(1→2) Man*p* residues. ManLAM is characterized by mono-, di, and tri-Man*p* caps. PIM_5 and PIM_6 contain α(1→2) Man*p* residues and mono- and di- Man*p* cap like structures, respectively.

***Reprinted with minor modifications from Kaur et al.10.1073/pnas.087761105. The scissors and dotted line indicate the cleavage of α(1→2) bonds causing the loss of mannose capping. The resultant structure represents our demannosylated lipoarabinomannan product.**