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: 70063219 **Manufacturing Date: 22AUG2023**

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 23.Rv.8.22.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

BEI Resources
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WORK SHEET FOR DEMANNOSYLATED LIPOARABINOMANNAN

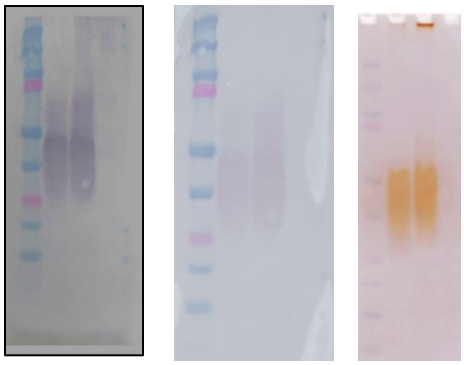
General Information
BEI Catalog Number: NR-56329
CSU Lot Number: 23.Rv.8.22.DLAM
Species: *Mycobacterium tuberculosis*
Strain: H37Rv

Purification Information
Cells Irradiated: Yes
Viability Test Performed: No Viable Organism Detected
LAM starting material (lot #): 23.Rv.03.23.LAM
LAM starting material (mg): 18.94 mg
Protocol used (SOP #): PP059 and SP079
Date started: 6/21/23
Date completed: 8/22/23
Notebook; pages Megan Stookey NB 3 pgs: 31-33, 43, 45, 46, 48-50, 53, 55, 56, 59-62, 64, 65, 68, 69, 72-75, 77, 78, 79, 81
Additional notes (if applicable): N/A

Quality Control Information
BCA: 0.021 mg protein per mg LAM — Notebook and page(s): Megan Stookey Notebook 3 pg: 77
Endotoxin assay used: EndoZyme II
Endotoxin amount: 1.114 ng/mg LAM — Notebook and page(s): Megan Stookey Notebook 3 pg: 73, 77
Image J Concentration: 1.646 mg/ml — Notebook and page(s): Megan Stookey Notebook 3 pg: 74, 75
Total amount of LAM: 5.082 mg
Silver stain date: 8/17/23 — Notebook and page(s): Megan Stookey Notebook 3 pg: 78,79
Western blot: 8/17/23 Antibody used: CS-35 — Notebook and page(s): Megan Stookey Notebook 3 pg: 78,79
Western blot: 8/17/23 Antibody used: Con A — Notebook and page(s): Megan Stookey Notebook 3 pg: 78,79
Amount Loaded on gel/s: 5 µg

QC Gel and Blots:

CS-35 Con A Silver



CS-35 and Con A:
Lane 1= Ladder
Lane 2= Demannosylated LAM product
Lane 3= H37Rv LAM
Lane 4= HSPX (negative control)

Silver Stain:
Lane 1=Ladder
Lane 2= Demannosylated LAM product
Lane3=H37Rv LAM

Aliquot Information:
*Aliquot information reflects those made at the time of QC. Bulk aliquots will be broken down as needed.

20 vials x 0.25 mg aliquots
1 vial x 0.082 mg aliquot

Megan Stookey 8/25/23
Research Associate Date

Rebecca Staudenmaier 8/25/2023
Laboratory Supervisor Date