

# Product Information Sheet 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.

**For research use only. Not for use in humans.**

#### **Contributor:**

BEI Resources or NIH - TB Vaccine Testing and Research Materials Contract

#### **Manufacturer:**

Karen Dobos, Ph.D., Colorado State University, Fort Collins, Colorado, USA or NIH - TB Vaccine Testing and Research Materials Contract

#### **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.<sup>1</sup> 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*.<sup>1,2</sup> 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.

The culture was grown to late log phase in glycerol-alanine-salts medium and washed with PBS. The cells were delipidated and suspended in PBS buffer containing 8% Triton X-114 and broken by French Press. The lysate was incubated at 4°C for 18 hours with rocking and insoluble material was removed by 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 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. Contaminating LPS is avoided, as all buffers and water used are endotoxin-free. Enzymatic demannosylation of the purified LAM by alpha-mannosidase derived from *Canavalia ensiformis* (Jack Bean) was performed by incubation in digest buffer (0.02 M sodium acetate, 5 mM zinc; pH 4.5) followed by digestion with proteinase K, ultrafiltration and dialysis in distilled, deionized water.

#### **Material Provided:**

Each vial contains approximately 250 µg of lyophilized, purified DLAM from *M. tuberculosis*, strain H37Rv.

**Note:** LAM can be reconstituted in water. A 100 mM to 500 mM aqueous buffered salt solution, such as phosphate buffered saline, may also be used.

#### **Packaging/Storage:**

NR-56329 was packaged aseptically in cryovials. The product is provided frozen on blue ice and should be stored at -80°C or colder immediately upon arrival. Freeze-thaw cycles should be avoided.

#### **Citation:**

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Mycobacterium tuberculosis*, Strain H37Rv, Purified Demannosylated Lipoarabinomannan (DLAM), NR-56329."

#### **Biosafety Level: 1**

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. [Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)](#), 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

#### **Disclaimers:**

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#### References:

1. Källénus, G., et al. "Lipoarabinomannan, and Its Related Glycolipids, Induce Divergent and Opposing Immune Responses to *Mycobacterium tuberculosis* Depending on Structural Diversity and Experimental Variations." *Tuberculosis (Edinb.)* 96 (2016): 120-130. PubMed: 26586646.
2. Kaur, D., et al. "A Single Arabinan Chain is Attached to the Phosphatidylinositol Mannosyl Core of the Major Immunomodulatory Mycobacterial Cell Envelope Glycoconjugate, Lipoarabinomannan." *J. Biol. Chem.* 289 (2014): 30249-30256. PubMed: 25231986.
3. Chatterjee, D., et al. "Lipoarabinomannan. Multiglycosylated Form of the Mycobacterial Mannosylphosphatidylinositols." *J. Biol. Chem.* 267 (1992): 6228-6233. PubMed: 1556131.
4. Chatterjee, D., et al. "Lipoarabinomannan of *Mycobacterium tuberculosis*. Capping with Mannosyl Residues in Some Strains." *J. Biol. Chem.* 267 (1992): 6234-6239. PubMed: 1556132.
5. Cole, S. T., et al. "Deciphering the Biology of *Mycobacterium tuberculosis* from the Complete Genome Sequence." *Nature* 393 (1998): 537-544. PubMed: 9634230. Erratum in: *Nature* 396 (1998): 190-198.

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