

***Mycobacterium smegmatis*, Purified Lipoarabinomannan (LAM)**

Catalog No. NR-14849

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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-14849 is a preparation of lipoarabinomannan (LAM) derived from the cell wall of *Mycobacterium smegmatis*. LAM possesses many biological activities including immunogenicity, induction of TNF and the release of other cytokines, and inhibition of antigen processing.

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 and suspended in PBS buffer containing 4% 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.

Material Provided:

Each vial contains approximately 500 µg of lyophilized purified LAM from *Mycobacterium smegmatis*. **Lyophilized products may aggregate as a loose powder near the lid. Please take precautionary measures (such as tapping the bottom of the tube on the lab bench) to reduce escape of the reagent when opening the vial to reconstitute contents.**

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-14849 was packaged aseptically in cryovials. The product 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 smegmatis*, Purified Lipoarabinomannan (LAM), NR-14849.”

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. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmb15/index.htm.

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References:

1. Chatterjee, D., et al. “Lipoarabinomannan. Multiglycosylated Form of the Mycobacterial Mannosylphosphatidylinositols.” J. Biol. Chem. 267 (1992): 6228-6233. PubMed: 1556131.

2. Chatterjee, D., et al. "Lipoarabinomannan of *Mycobacterium tuberculosis*. Capping with Mannosyl Residues in Some Strains." J. Biol. Chem. 267 (1992): 6234-6239. PubMed: 1556132.
3. 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.
4. Khoo, K. H., et al. "Truncated Structural Variants of Lipoarabinomannan in Ethambutol Drug-Resistant Strains of *Mycobacterium smegmatis*." J. Biol. Chem. 271 (1996): 28682-28690. PubMed: 8910503.

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