

Certificate of Analysis for NR-19328

Mycobacterium leprae Cytosol Fraction (MLSA) minus LAM

Catalog No. NR-19328

This reagent is the tangible property of the U.S. Government.

Product Description: NR-19328 was produced from purified *Mycobacterium leprae* (*M. leprae*) whole cells (non-irradiated, non-autoclaved) extracted from a pool of infected armadillo liver and spleen tissue. Cells were sonicated for forty, one-minute cycles and an acid fast stain of the sonicate was performed to confirm complete breakage of the cells. The sonicate was centrifuged to pellet the cell wall material and the resulting supernatant was further centrifuged to pellet the cell membrane material. The LAM was removed from the cytosolic protein fraction by TX-114 extraction. The final supernatant was collected and the protein content quantitated by BCA assay.

Lot: 61181930 Manufacturing Date: 30OCT2012

Production and QC testing were performed by Colorado State University. The Colorado State University documentation for lot MLSAminusLAM.Batch103.08.23.2012.JNK 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[®] 3 knowledge.

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BEI Resources www.beiresources.org E-mail: contact@beiresources.org
Tel: 800-359-7370

Fax: 703-365-2898

QC SHEET FOR M. LEPRAE MLSA CYTOSOL MINUS LAM PROTEIN

General Information

Product Lot Number: MLSAminusLAM.Batch103.08.23.2012.JNK

Catalog Number: NR-19328 Species: Mycobacterium leprae Strain: NHDP-55

Cell prep pool:

	750					
Armadillo	3T50	liver	NHDP-55	harvested	02.02.2011	4.51mg
Armadillo	5L55	spleen	NHDP-55	harvested	03.15.2012	15.67mg
Armadillo	5L55	liver	NHDP-55	harvested	07.05.2012	24.65mg
Armadillo	5L55	liver	NHDP-55	harvested	07.08.2012	14.73mg
Armadillo	5L55	liver	NHDP-55	harvested	07.10.2012	23.11mg
Armadillo	5L55	liver	NHDP-55	harvested	07.12.2012	22.91mg
Armadillo	5L55	liver	NHDP-55	harvested	07.15.2012	16.71mg
Armadillo	5L55	liver	NHDP-55	harvested	07.17.2012	26.42mg
Armadillo	5L55	liver	NHDP-55	harvested	07.19.2012	12.65mg
Armadillo	5L55	spleen	NHDP-55	harvested	07.22.2012	37.32mg

Sonication and Fractionation Information

Starting material (lot #):	Batch #103 Cell amount (wet weight):198.68 mg whole cells				
SOP #: subcellular frac	tionation protocol, LAM Removal protocol, and PP010 Notebook pages: Tissue				
Prep Book#3 p31-33	Date of Probe Sonication: 08/21/12 Buffer used: PBS				
Centrifugation Notes:	27,000 x g spin to obtain supernatant (cytosol plus membrane); 75,000 x g spin				
of this to obtain supernatant fraction (MLSA).					

Quality Control Information

Clarity of product: <u>orange/amber in color</u> BCA: <u>2.4 µg/µl</u> Notebook and page(s): <u>JNK notebook #3 pp.</u> 21-27 Total volume: 3 ml Total amount of Protein: 7.2 mg Silver Stain Date: 10/22/12 Western blot: 4μg MLSA per lane, α-MLSA-LAM (pAb), 1:5,000 culture supernatant.

QC Gel and Western blot:

Silver Stain (w/ periodic acid)

Lane 1- Molecular Weight

Lane 2- MLSA Batch #102 (2ug)

Lane 3- MLSA-LAM Batch #103 (2ug)

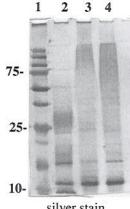
Lane 4- MLSA-LAM Batch #80 (2ug)

Western Blot

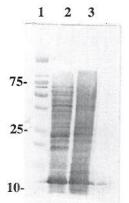
Lane 1- Molecular Weight

Lane 2- MLSA-LAM Batch#103

Lane 3- MLSA-LAM Batch#80







Western Blot

10/21/2012

Aliquot Information: 0.25mg x 24 vials lyophilized

al Supervisor / Date