

Certificate of Analysis for NR-19333

Mycobacterium leprae SDS-Soluble Cell Wall Fraction (MLCwA)

Catalog No. NR-19333

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

Product Description: NR-19333 was produced from purified *Mycobacterium leprae* liver and spleen whole cells (non-irradiated, non-autoclaved) extracted from a pool of infected armadillo 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 which was washed three times with phosphate buffered saline to remove any contaminating cytosolic or membrane proteins. The washed pellet was resuspended in phosphate buffered saline and 2% sodium dodecyl sulfate (SDS) and heated. After one hour the mixture was centrifuged to remove the insoluble material. After two additional extractions the extracts were pooled and passed over an Extractigel-D column to remove the SDS. The final preparation was quantitated for protein content by BCA assay.

Lot: 61391641 Manufacturing Date: 21AUG2012

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot *M.lep*.MLCwA.103.08.21.2012.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® & knowledge.

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QC SHEET FOR M. LEPRAE SOLUBLE CELL WALL PROTEIN (MLCwA)

General Information

Product Lot Number: M. lep MLCwA.103.08.21.2012.JNK

Species: Mycobacterium leprae

Strain: NHDP-55
BEI Catalog Number: NR-19333

Cell prep pool:

| Com brok book | . • | | | | | |
|---------------|------|--------|---------|-----------|------------|---------|
| 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): 198.68mg whole cells

SOP #: <u>subcellular fractionation protocol</u> Notebook pages: <u>Tissue Prep#3 pp31-33</u>

Date of Probe Sonication: 08/21/2012 Buffer used: PBS

Centrifugation Notes 27,000 x g spin to obtain cell wall pellet; washed cell wall extracted with 2%

SDS at 55C three times, pooled; detergent removed by SDS Removal Protocol.

Quality Control Information

Clarity of product: __cream

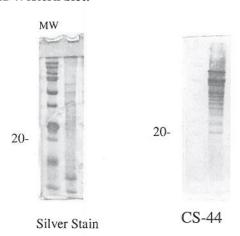
BCA: 37.8µg/µl Notebook and page(s): Tissue Prep Boook#3pp36-37

Total volume: 1.110 ml Total amount of Protein: 39.75 mg

Silver Stain Date: __11/14/2012 Western blot: __4 µg MLCwA per lane, CS-44 (mAb to GroEL2,

ML0317), 1:20 culture supernatant

QC Gel and Western blot:



Aliquot Information: 7ul/vial, ~0.26mg/ vial x 159 vials, frozen and lyophilized.

Produced by/ Date

Produced by/ Date

Lab Supervisor / Date

2 /2/18/2012