

Certificate of Analysis for NR-36503

Mycobacterium tuberculosis, Strain East African Indian 91_0079, Cell Membrane Fraction

Catalog No. NR-36503

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

Product Description: NR-36503 is a preparation of the cell membrane fraction of *Mycobacterium tuberculosis*, strain East African Indian 91_0079, and contains the cytoplasmic membrane and components of the outer lipid layer. The culture was grown to late log phase in glycerol-alanine-salts medium, washed with PBS, and inactivated by gamma irradiation. The bacilli were broken in a French Press pressure cell and unbroken cells were removed by low speed centrifugation. The cell wall was isolated by centrifugation at 27,000 x g. The supernatant was subjected to a 100,000 x g centrifugation for four hours and the resulting membrane pellet was washed with PBS, then suspended and dialyzed in 10 mM ammonium bicarbonate. Protein content was determined using the BCA protein assay.

Lot: 62575037 Manufacturing Date: 13MAY2014

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 14.EAI.5.14.MEM 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.

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WORK SHEET FOR MEMBRANE

General Information

BEI Catalog Number: 62575037

Product Lot Number: 14.EAI.5.14..MEM

Species: M. Tuberculosis

Strain: EAI

Sonication and Fractionation Information

Starting material (lot #): 12.EAI.7.23.6.WCg

Cell amount (wet weight): 41g

SOP #: PP008.1 Notebook pages: Rachel Hazenfield Protein #2 pp. 107-111

Date of French Press Sonication: 4/14/14; Buffer used: PBS-EDTA with protease inhibitor, DNase, and

RNase

Centrifugation Notes Centrifuged at 100,000 x g to recover from cytosol

Date 10 mM ammonium bicarbonate dialysis started: 5/12/14

date completed: 05/13/13/14

Notebook; page(s): Rachel Hazenfield Protein #2 pg.109

Quality Control Information

Clarity of product/suspension after dialysis: Milky-beige (normal)

BCA: <u>0.815</u> μg /μl.

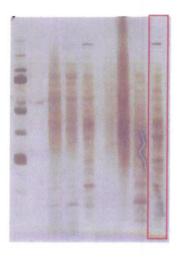
Notebook and page(s): Rachel Hazenfield Protein #2 pg.111

Total volume: 19.509ml Total amount of Protein: 15.9mg

Silver Stain Date: 5/14/14.

Notebook and page(s): Rachel Hazenfield Protein #2 pg. 110

QC Gel:



Aliquot Information: 15 x 1 mg; 1 x 0.9mg

Research Associate) (date)

(Laboratory Supervisor)

(date)