

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: 61449190 Manufacturing Date: 01DEC2012

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 12.EAI.11.20.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[®] 3 knowledge.

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WORK SHEET FOR MEMBRANE

General Information

Product Lot Number: 12.EAI.11.20.MEM

Species: M. tuberculosis

Strain: EAI

Sonication and Fractionation Information

Starting material (lot #): 12.EAI.9.17.10.WCg, 12.EAI.8.6.7.WCg, & 12.EAI.9.4.9.WCg

Cell amount (wet weight): 84.25g (Only ~42g used for this product)
SOP #: PP008.1 Notebook pages: Rachel Hazenfield Protein #1 pp. 98-100

Date of French Press Sonication: 11/20/2012; 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: 11/29/2012

date completed: 12/01/2012

Notebook; page(s): Rachel Hazenfield Protein #1 pp. 99-100

Quality Control Information

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

BCA: 0.921 µg /µl. Notebook and page(s): Rachel Hazenfield Protein #1 pg. 99

Total volume: 8.252 ml Total amount of Protein: 7.6 mg

Silver Stain Date: 12/04/2012. Notebook and page(s): Rachel Hazenfield Protein #1 pg. 100

QC Gel:



Aliquot Information:

*Aliquot information reflects aliquots made at the time of QC. Bulk aliquots will be broken down as needed.

7x 1 mg; 1 x 0.6 mg

(date) (Laboratory Supervisor)

(date)