

Mycobacterium tuberculosis*, Strain East African Indian 91_0079, Cell Wall Fraction*Catalog No. NR-36500**

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Product Description: NR-36500 is a preparation of the cell wall fraction of *Mycobacterium tuberculosis*, strain East African Indian 91_0079, and contains proteins and non-protein compounds such as mAGP. The culture was grown to late log phase in glycerol-alanine-salts medium, washed with PBS pH 7.4, and inactivated by gamma irradiation. The bacilli were suspended at a concentration of 2 g/mL in PBS containing 8 mM EDTA, DNase, RNase, and a proteinase inhibitor tablet, and broken in a French Press pressure cell at 4°C. Unbroken cells were removed by low speed (3,000 x g) centrifugation. The cell wall was isolated by centrifugation at 27,000 x g for one hour and washed two times in PBS. The final cell wall pellet was suspended and dialyzed in 10 mM ammonium bicarbonate, quantified by BCA protein assay for protein content, and frozen.

Lot: 61449187**Manufacturing Date: 01DEC2012**

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 12.EAI.11.20.CW is attached.

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WORK SHEET FOR CELL WALL

General Information

Product Lot Number: 12.EAI.11.20.CW

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 27,000 x g to separate from membrane and 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, off-white (normal)

BCA: 10.291 $\mu\text{g}/\mu\text{l}$.

Notebook and page(s): Rachel Hazenfield Protein #1 pg. 99

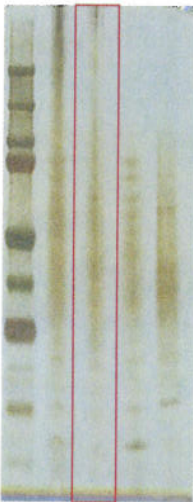
Total volume: 110 ml

Total amount of Protein: 1029.1 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.

100 x 1 mg; 1 x 929.1mg

Rachel Hazenfield 12-5-12
(Research Associate) (date)

[Signature] 12/5/2012
(Laboratory Supervisor) (date)