

Certificate of Analysis for NR-14828

Mycobacterium tuberculosis, Strain H37Rv, Cell Wall Fraction

Catalog No. NR-14828

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

Product Description: NR-14828 is a preparation of the cell wall fraction of *Mycobacterium tuberculosis*, strain H37Rv 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 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 centrifugation. The cell wall was isolated by centrifugation at 27,000 x g for one hour and washed in PBS. The final cell wall pellet was suspended and dialyzed in 0.01 M ammonium bicarbonate. Protein content was determined using the BCA protein assay.

Lot: 08.Rv.2.5.21.7.CW Manufacturing Date: 13AUG2008

QC testing was performed by Colorado State University under the TB Vaccine Testing and Research Materials Contract (NIH). The Colorado State University documentation 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 CELL WALL

General Information

Product Lot Number: 08.Rv.2.5.21.7.CW Species: <u>Mycobacterium tuberculosis</u>

Strain: H37Rv

Sonication and Fractionation Information

Starting material (lot #): 08.Rv.2.5.21.7.WCg

Cell amount (wet weight): 103g

SOP #: PP008.1 Notebook pages: Agatha #1 pp. 78-80

Date of French Press Sonication: 8/11/08;

Buffer used: Breaking Buffer: 500ml PBS, 1.0ml 0.5M EDTA, 60ul DNase, 60ul RNase, 2 Tablets

Protease Inhibitors

Centrifugation Notes 27,000xg, 4°C, 1hr; 27,000xg, 4°C, 20min; 100,000xg, 4°C, 4hrs (x2)

Date 10 mM ammonium bicarbonate dialysis started: 8/12/08

date completed: 8/13/08

Notebook; page(s): Agatha #1 pp. 78-79

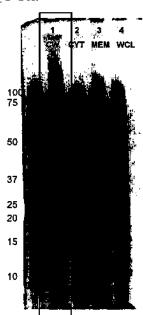
Quality Control Information

Clarity of product/suspension after dialysis: Cloudy brown

BCA: 13.44 μg /μl. Notebook and page(s): Agatha #1 pg. 79
Total volume: 152.5ml Total amount of Protein: 2,050 mg

Silver Stain Date: 8/13/08. Notebook and page(s): Agatha #1 pg. 80

QC Gel:



Aliquot Information:

50 x 1mg 40 x 50mg

Haft Diecyolek 8/18/08
(Kesearch Associate) (date)

(Laboratory Supervisor) (date)