

Mycobacterium tuberculosis*, Strain H37Rv, Cell Wall Fraction*Catalog No. NR-14828**

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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: 09.Rv.2.5.22.5.CW**Manufacturing Date: 10SEP2009**

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.

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You are authorized to use this product for research use only. It is not intended for human use.



HHSN266200400091C

WORK SHEET FOR CELL WALL

General Information

Product Lot Number: 09.Rv.2.5.22.5.CW
Species: M. tuberculosis
Strain: H37Rv

Sonication and Fractionation Information

Starting material (lot #): 09.Rv.2.5.22.5.WCg
Cell amount (wet weight): 97g
SOP #: PP008.1 Notebook pages: Megan Lucas, Contract Subcellular Fractions; pg 31-34
Date of French Press Sonication: 08 / 14 / 2009; Buffer used: 1mM EDTA with DNase, RNase, and Complete protease inhibitor (later dialyzed into PBS)
Centrifugation Notes 3500 rpm, 5 minutes (08/25/09); 27000xg, 2 x 1 hour (08/25/09 and 08/26/09)
Date 10 mM ammonium bicarbonate dialysis started: 09 / 08 / 2009
date completed: 09 / 10 / 2009
Notebook; page(s): MCL, Contract Subcellular Fractions; pg 32

Quality Control Information

Clarity of product/suspension after dialysis: Uniform suspension
BCA: 14.25 $\mu\text{g} / \mu\text{l}$ Notebook and page(s): MCL, Contract Subcellular Fractions; pg 33
Total volume: 113.7 ml Total amount of Protein: 1620 mg
Silver Stain Date: 09 / 17 / 2009 Notebook and page(s): MCL, Contract Subcellular Fractions; pg 34

QC Gel:



Aliquot Information:

20 x 1 mg
32 x 50 mg

Megan Lucas 9-18-09
(Research Associate) (date)

[Signature] 9/18/2009
(Laboratory Supervisor) (date)