

***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. 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 are 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 are removed by low speed (3,000 x g) centrifugation. The cell wall is isolated by centrifugation at 27,000 x g for one hour and washed two times in PBS. The final cell wall pellet is suspended and dialyzed in 0.01 M ammonium bicarbonate, quantified by BCA protein assay for protein content, and frozen.

Lot^{1,2}: 63906064

Manufacturing Date: 04SEP2011

TEST	SPECIFICATIONS	RESULTS
SDS-PAGE and Silver Stain ²	Report results	Visible smear of cell wall proteins (Figure 1)
Concentration by Bicinchoninic Acid Protein Assay ^{2,3}	Report results	14.89 µg/µL
Quantity per vial	~ 1 mg	~ 1 mg
Volume per vial	Report results	~ 70 µL

¹Production was performed by Colorado State University (CSU) and provided as a bulk lot. NRC-14828 bulk lot 10.Rv.2.11.8.6.CW was aliquoted at ATCC® on 03DEC2015 as NR-14828 lot 63906064. Testing by BEI Resources was performed on NR-14828 lot 63906064 after vialing. The CSU documentation for bulk lot 10.Rv.2.11.8.6.CW is attached.

²Retesting of NR-14828 lot 63906064 was performed by BEI Resources in October 2016 to confirm product stability.

³Using BSA as a standard

Date: 06 DEC 2016

Signature:



BEI Resources Authentication

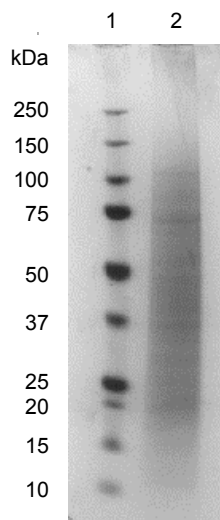
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Figure 1: SDS-PAGE of Cell Wall Fraction



Lane 1: Precision Plus Protein™ Standard

Lane 2: NR-14828 lot 63906064, 6.0 µg

WORK SHEET FOR CELL WALL

General Information

Product Lot Number: 10.Rv.2.11.8.6.CW

Species: *Mycobacterium tuberculosis*

Strain: H37Rv

Sonication and Fractionation Information

Starting material (lot #): 10.Rv.2.11.8.6.wcg

Cell amount (wet weight): 75.7g

SOP #: PP007.1 Notebook pages: Nick May #48

Date of French Press Sonication: 08 / 29 / 11; Buffer used: 1mM EDTA-PBS

Centrifugation Notes Centrifuged at 27,000 x g to separate from membrane and cytosol.

Date 10 mM ammonium bicarbonate dialysis started: 08 / 31 / 11

date completed: 09 / 04 / 11

Notebook; page(s): Prucha #109

Quality Control Information

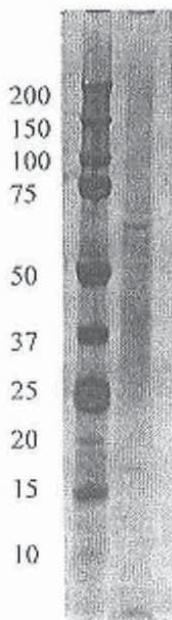
Clarity of product/suspension after dialysis: Creamy, off white (normal)

BCA: 13.64 $\mu\text{g}/\mu\text{l}$. Notebook and page(s): Prucha #110

Total volume: 100 ml Total amount of Protein: 1364 mg

Silver Stain Date: 09 / 08 / 11 Notebook and page(s): Prucha #110

QC Gel:



Aliquot Information:

None

C. Melchior
(Research Scientist) 9/20/11
(date)

K. R. B.
(Laboratory Supervisor) 9/20/11
(date)