

Mycobacterium tuberculosis*, Strain HN878, Cell Wall Fraction*Catalog No. NR-14830**

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

Product Description: NR-14830 is a preparation of the cell wall fraction of *Mycobacterium tuberculosis*, strain HN878 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 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 0.01 M ammonium bicarbonate, quantified by BCA protein assay for protein content, and frozen.

Lot: 61190399**Manufacturing Date: 13AUG2011**

QC testing was performed by Colorado State University under the TB Vaccine Testing and Research Materials Contract (NIH). The Colorado State University documentation for lot 10.HN878.2.25.5.CW 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.

ATCC[®] is a trademark of the American Type Culture Collection.

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



WORK SHEET FOR CELL WALL

General Information

Product Lot Number: 10.HN878.2.25.5.CW

Species: *Mycobacterium tuberculosis*

Strain: HN878

Sonication and Fractionation Information

Starting material (lot #): 10.HN878.2.25.5.wcg

Cell amount (wet weight): 43.8g

SOP #: PP007.1 Notebook pages: Nick May

Date of French Press Sonication: 07 / 26 / 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 / 10 / 11

date completed: 08 / 13 / 11

Notebook; page(s): Prucha #106

Quality Control Information

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

BCA: 3.08 $\mu\text{g} / \mu\text{l}$.

Notebook and page(s): Prucha #108

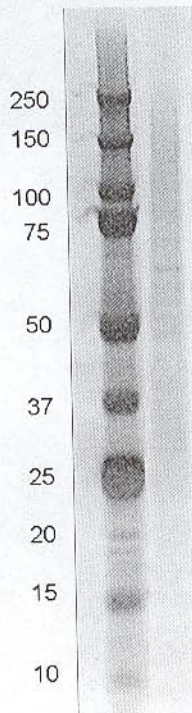
Total volume: 15 ml

Total amount of Protein: 46.2 mg

Silver Stain Date: 08 / 16 / 11

Notebook and page(s): Prucha #108

QC Gel:



Aliquot Information:

None

C. McChaffy
(Research Scientist)

8-29-11
(date)

[Signature] 8/30/11
(Laboratory Supervisor) (date)