

# Certificate of Analysis for NR-14829

## Mycobacterium tuberculosis, Strain CDC1551, Cell Wall Fraction

## Catalog No. NR-14829

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

**Product Description:** NR-14829 is a preparation of the cell wall fraction of *Mycobacterium tuberculosis*, strain CDC1551 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: 61190402 Manufacturing Date: 14SEP2012

QC testing was performed by Colorado State University under the TB Vaccine Testing and Research Materials Contract (NIH). The Colorado State University documentation for the lot 12.CSU93.8.7.CW is attached.

ATCC<sup>®</sup>, 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<sup>®</sup>'s knowledge.

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#### WORK SHEET FOR CELL WALL

#### **General Information**

Product Lot Number: 12.CSU93.8.7.CW

Species: M. tuberculosis
Strain: CDC1551

#### Sonication and Fractionation Information

Starting material (lot #): 10.CSU93.8.23.6.WCg & 06.CSU93.11.17.8.WCg.IHO

Cell amount (wet weight): 75g (Only ~37g used for this product)

SOP #: PP008.1 Notebook pages: Rachel Hazenfield Protein #1 pp. 55-59

Date of French Press Sonication: 08/07/2012 and 09/05/2012; 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: 09/11/2012 date completed: 09/14/2012

Notebook; page(s): Rachel Hazenfield Protein #1 pg. 56

### **Quality Control Information**

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

BCA: 11.368 μg /μl. Notebook and page(s): Rachel Hazenfield Protein #1 pg. 57

Total volume: 59.4 ml Total amount of Protein: 675.24 mg

Silver Stain Date: 09/25/2012. Notebook and page(s): Rachel Hazenfield Protein #1 pg. 59

QC Gel:



#### Aliquot Information:

\*Aliquot information reflects aliquots made at the time of QC. Bulk aliquots will be broken down as needed.

50 x 1 mg; 1 x 625.24mg

(Laboratory Supervisor)

(date)