

Certificate of Analysis for NR-14836

Mycobacterium tuberculosis, Strain HN878, Cytosol Fraction

Catalog No. NR-14836

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

Product Description: NR-14836 is a preparation of the cytosol fraction of *Mycobacterium tuberculosis*, strain HN878, and contains cytosolic proteins and soluble material released from the cell wall during disruption of the bacilli.

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 (3,000 x g) centrifugation. The cell wall was isolated by centrifugation at 27,000 x g. The supernatant was subjected to a 100,000 x g centrifugation for four hours, then collected and dialyzed against 0.01 M ammonium bicarbonate. The protein content was determined using the BCA protein assay.

Lot: 61190401 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.CYT 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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NR-14836_61190401_28SEP2012

WORK SHEET FOR CYTOSOL

General Information

Product Lot Number: 10.HN878.2.25.5.CYT

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 #45

Date of French Press Sonication: 07 / 26 / 11; Buffer used: 1mM EDTA-PBS

Centrifugation Notes Centrifuged two times at 100,000 x g to separate from membrane_

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: _____ Clear

BCA: 2.85 µg /µl. Notebook and page(s): Prucha #109

Total volume: 90 ml Total amount of Protein: 256.5 mg
Silver Stain Date: 08 /16 /11 Notebook and page(s): Prucha #108

QC Gel:

Aliquot Information:

1 x 50ml 1 x 40ml

C. Meliaffy 8-29-11
Research Scientist (date)

(Laboratory Supervisor)

(date)