

Mycobacterium tuberculosis*, Strain CDC1551, Cytosol Fraction*Catalog No. NR-14835**

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

Product Description: NR-14835 is a preparation of the cytosol fraction of *Mycobacterium tuberculosis*, strain CDC1551, 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 10 mM ammonium bicarbonate. The protein content was determined using the BCA protein assay.

Lot: 61216440**Manufacturing Date: 18MAR2005**

QC testing was performed by Colorado State University under the TB Vaccine Testing and Research Materials Contract (NIH). The Colorado State University documentation for bulk lot 05.CS.93.1.12.5.CYT is attached. This lot was aliquoted at ATCC®.

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



WORK SHEET FOR CYTOSOL

General Information

Product Lot Number: 05.CS.93.1.12.5.CYT
Species: M. tuberculosis
Strain: CSU93

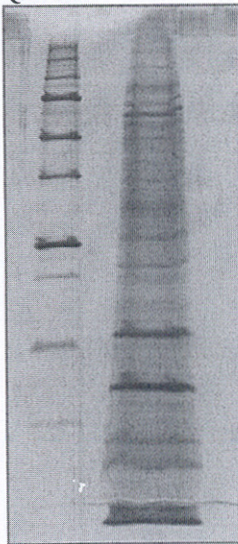
Sonication and Fractionation Information

Starting material (lot #): 05.CSU93.1.12.5.yWC
Cell amount (wet weight): 47.25g (half of a 94.5g batch)
SOP #: PP008 Notebook pages: Megan Lucas, Contract Subcellular Fractions; pg 20-22
Date of French Press Sonication: 03 / 03 / 2005; Buffer used: PBS+EDTA, DNase, RNase, and protease inhibitors
Centrifugation Notes 3000xg, 15min (3/3/05); 27000xg, 1hr (3/8/05); 100000xg, 4hr (3/9/05 & 3/15/2004)
Date 10 mM ammonium bicarbonate dialysis started: 03 / 15 / 2005
date completed: 03 / 18 / 2005
Notebook; page(s): MCL, Contract Subcellular Fractions; pg 21-22

Quality Control Information

Clarity of product/suspension after dialysis: clear brown
BCA: 5.32 $\mu\text{g} / \mu\text{l}$. Notebook and page(s): MCL, Contract Subcellular Fractions; pg 22
Total volume: 130.6 ml Total amount of Protein: 695 mg
Silver Stain Date: 03 / 18 / 2005 Notebook and page(s): MCL, Contract Subcellular Fractions; pg 22

QC Gel:



Aliquot Information:

13 x 50 mg
3 x 5 mg
30 x 1 mg

Megan Lucas 3-21-05
(Research Associate) (date)

[Signature] 3/21/2005
(Laboratory Supervisor) (date)