

Certificate of Analysis for NR-14844

Mycobacterium tuberculosis, Strain H37Rv, Purified Trehalose Dimycolate (TDM)

Catalog No. NR-14844

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

Product Description: NR-14844 is a preparation of purified trehalose dimycolate (TDM) that was extracted from *Mycobacterium tuberculosis*, strain H37Rv cells with chloroform/methanol (2:1) and purified on a silica gel column. The column was washed with chloroform and the TDM fraction was eluted with 5% methanol in chloroform. The eluted TDM was further purified on a C18 reverse phase Sep Pak filter and eluted with 60% chloroform in methanol. The purified TDM was completely dried under nitrogen gas.

Lot: 61773629 Manufacturing Date: 31JAN2012

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for pre-vial bulk lot 11.Rv.1.27.01.TDM is attached. This lot was received as a dry pellet from CSU and was rehydrated in chloroform:methanol (2:1) at ATCC. Approximately 1 mL of a 250 µg/mL solution was dispensed per vial and lyophilized yielding a final amount of 250 µg per vial.

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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WORK SHEET FOR PURIFIED TDM

General Information

Lot Number: _	11.Rv.1.27.01.TDM	
Species:	M. tuberculosis	
Strain:	H37Rv	

Purification Information

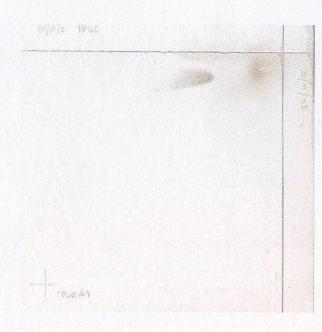
Starting material:	2:1 chloroform/methanol total lipid Feb 2011
Lot number:	Whole cells 10.Rv.2.3.22.6.WCg
Protocol used (SOP #'s):	SP031, SP032, SP033, SP037, SP046, PP029a
Date started:	3/4/11
Date completed:	1/31/12
Notebook; page(s):	Lipids Notebook 4 pp 3-44

Additional notes: Preparatory plates were loaded with total lipid and developed with 65/25/4 chloroform/ methanol/water. Additional prep plates were used for further clean-up, according to PP029a. Total lipid was also applied to silica gel column, and both 5% and 10% MeOH washes, enriched in TDM, were likewise applied to prep plates.

Quality Control Information:

Total volume: _	2.16 ml	_ Total amount of TDM: 5.40 mg
TLC date:	1/9/12	Date dried on N ₂ bath: 1/31/12
Notebook and p	age(s): Lipids Note	ebook 4 pp 45-48

TLC Analysis:



TLC solvent system

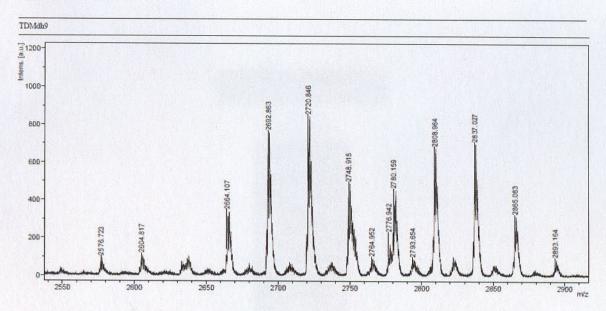
1st dimension 80/20/2 chloroform/ methanol/ water, bottom to top.

2nd dimension 100/14/0.8 chloroform/ methanol/ water, left to right.

Loaded approximately 25 µg TDM.

Aliquot information

1 x 5.40 mg



Purified sample (1 ul) was mixed 1:1 with DHB matrix and analyzed in negative electrospray mode.

(Research Associate)

date

5/1/12

(Laboratory Supervisor)

date