

Certificate of Analysis for NR-14845

Mycobacterium tuberculosis, Strain H37Rv, Purified Sulfolipid-1

Catalog No. NR-14845

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

Product Description: NR-14845 is a preparation of purified sulfolipid-1 (SL-1) that was extracted from *Mycobacterium tuberculosis*, strain H37Rv cells with chloroform/methanol (2:1) and purified on a silica gel column. The loaded column was washed with chloroform and the SL-1 fraction was eluted with 5% methanol in chloroform. The SL-1 fraction was further purified on a C18 reverse phase Sep Pak filter which was washed with 60% chloroform in methanol to remove the trehalose dimycolate fraction followed by elution of the purified SL-1 with 25% chloroform in methanol.

Lot: 62325995 Manufacturing Date: 30APR2014

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 14.Rv.4.28.01.SLdh9 is attached.

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WORK SHEET FOR PURIFIED SL-I

General Information

Lot Number: _	14.Rv.4.28.01.SLdh9	
Species:	M. tuberculosis	
Strain:	H37Rv	

Purification Information

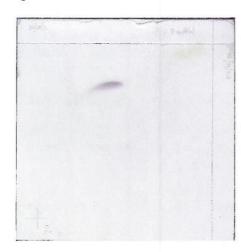
Starting material:	10:10:3 CHCl3/CH3OH/H2O-extracted total lipid Dec 2013	
Lot number:	Whole Cells 13.Rv.2.10.22.7.WCg a,b	
Protocol used (SOP #'s):	PP034, SP031, SP032, SP033, SP037	
Date started:	3/24/14	
Date completed:	4/30/14	
Notebook; page(s):	TDM & SL Notebook 2 pp 59-70	

Additional notes: Preparatory TLC plates were loaded with 2:1-soluble 10:10:3 total lipid and developed with 100/14/0.8 chloroform/methanol/water. SL-bearing silica was extracted with 2:1 chloroform/methanol. A final clean-up with C18 columns was employed.

Quality Control Information

Total volume: 2.5 ml Total amount of SL-I: 6.25 mg Date dried on N_2 bath: 5/2/14 Notebook and page(s): $\overline{\text{TDM \& SL Notebook 2 pp 71-74}}$ TLC date: 4/28/14 MALDI-TOF date: 4/30/14

QC TLC

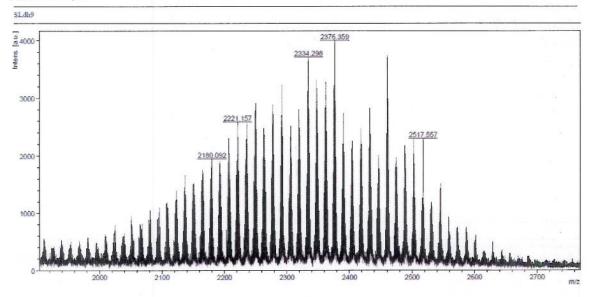


Ran 25 μ g SL. 1st dimension 100/14/0.8 CHCl₃/CH₃OH/H₂O (left \rightarrow right), 2nd dimension 80/20/2 CHCl₃/CH₃OH/H₂O (bottom \rightarrow top), stained with α -naphthol.

Aliquot Information

 $25 \times 0.25 \text{ mg} = 6.25 \text{ mg}$

MALDI-TOF



Mixed 1.0 μ l sulfolipid sample at 1.0 mg/ml in 2:1 CHCl₃/CH₃OH with 1.0 μ l DHAB matrix, and analyzed in negative electrospray mode with TFA.

Dan (Vo) esto

(Research Associate)

5/2/14

date

(Laboratory Supervisor)

date