

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 irradiated *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: 62575032 Manufacturing Date: 25JUL2014

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 14.Rv.7.23.02.SL 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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BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

WORK SHEET FOR PURIFIED SL-I

General Information

Lot Number: _	14.Rv.7.23.02.SL		
Species:	M. tuberculosis		
Strain:	H37Rv	77	- 8

Purification Information

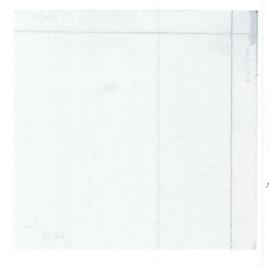
Starting material:	2:1 CHCl3/CH3OH-extracted total lipid May 2014
Lot number:	Whole Cells 13.Rv.2.3.19.8.WCg
Protocol used (SOP #'s):	PP034, SP031, SP032, SP033, SP037
Date started:	5/28/14
Date completed:	7/25/14
Notebook; page(s):	TDM & SL Notebook 2 pp 79-87

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

Quality Control Information

Total volume: 0.64 ml Total amount of SL-I: 1.6 mg Date dried on N_2 bath: 7/25/14 Notebook and page(s): 1.6 mg Date dried on N_2 bath: 1.6 mg Date dried on N_2 Date dried on

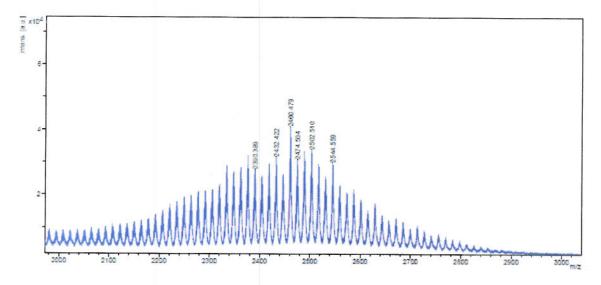
QC TLC



Ran 20 μg 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

6 x 0.25 mg = 1.5 mg 1 x 0.10 mg = $\frac{0.1 \text{ mg}}{1.6 \text{ mg}}$



Mixed 1.0 μ l (2.5 ug) sulfolipid sample in 2:1 CHCl₃/CH₃OH with 1.0 μ l DHAB matrix, and analyzed in negative electrospray mode with TFA.

Day Waser -1/25/14

(Research Associate)

date

(Laboratory Supervisor)

date