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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-44101

Mycobacterium tuberculosis, Strain H37Rv, Mycobactin (MBT)

Catalog No. NR-44101
This reagent is the tangible property of the U.S. Government.

Product Description: NR-44101 is a preparation of mycobactin (MBT) derived from *Mycobacterium tuberculosis* (*M. tuberculosis*), strain H37Rv. Mycobactin, an iron-binding siderophore, is located in the cell wall where it facilitates the transport of iron to the interior of the cell. Mycobactin may also function as a growth factor in low-iron environments and has demonstrated a possible role in microbial virulence.

Lot: 61983709 **Manufacturing Date: 16MAY2014**

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 14.Rv.05.12.1.MBT 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.

Support Provided by NIAID

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WORK SHEET FOR PURIFIED MYCOBACTIN

General Information

Lot Number: 14.Rv.05.12.1.MBT
Species: *M. tuberculosis*
Strain: H37Rv

Purification Information

Starting material: 10:10:3 TL and EtOH TL
Lot number: 13.Rv.2.10.22.7.WCg a,b and 13.Rv.2.7.9.5.WCg
Protocol used (SOP #'s): PP018.1, PP032.2, SP004, SP005, SP031b, SP032, SP033
Date started: 1/15/14
Date completed: 5/16/14
Notebook; page(s): Mycobactin Notebook II pp 59-115

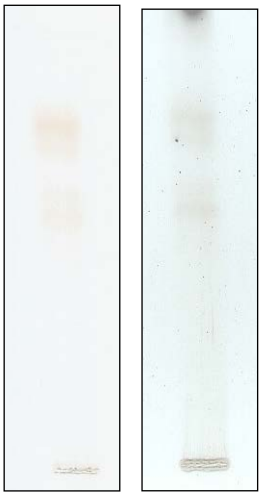
Additional notes: 10:10:3TL or ethanol-extracted TL from H37Rv was developed on preparatory TLC plates with 95/5 chloroform/ methanol, and the resultant extracts further purified by several rounds of prep plate development with 2/3/3 petroleum ether/ n-butanol/ ethyl acetate.

Quality Control Information

Total volume: 2.9 ml Total mycobactin: 2.9 mg Date dried on N_2 bath: 5/16/14
Notebook and page(s): Mycobactin III pp 1-7 TLC date: 5/15/14 MALDI-TOF date: 5/12/14

QC TLC

Unstained **$CuSO_4$**

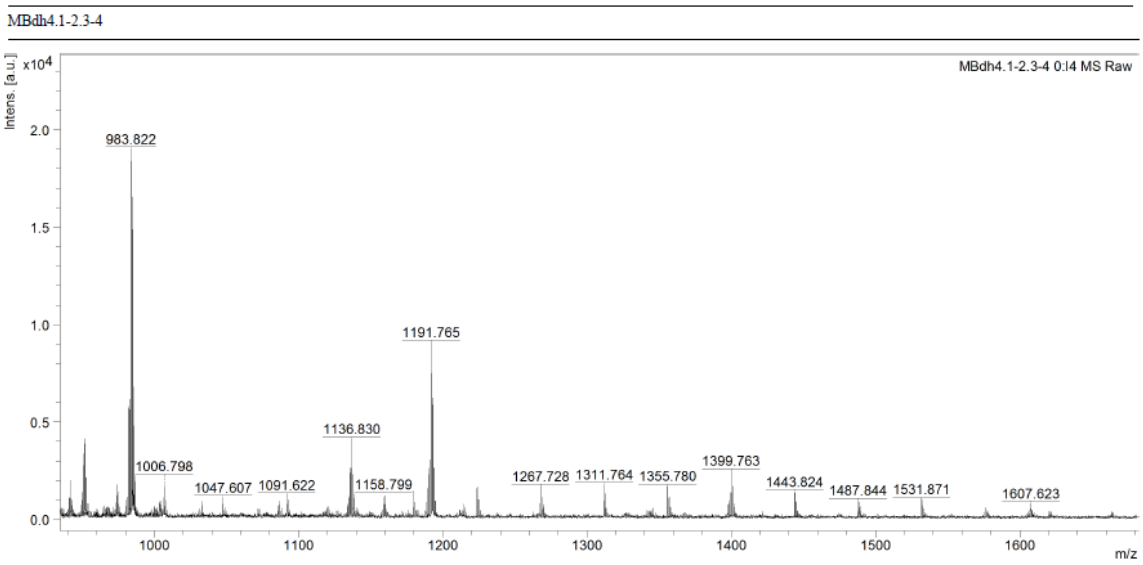


Developed 200 µg mycobactin in 2/3/3 petroleum ether/ n-butanol/ ethyl acetate.

Aliquot Information

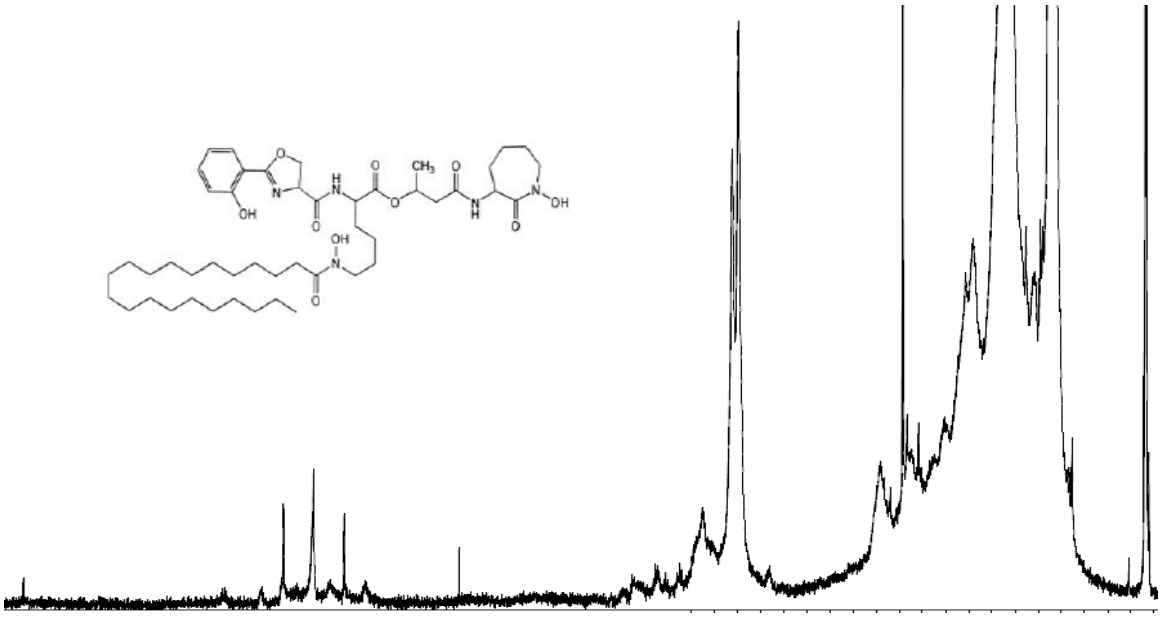
25 x 100 µg = 2500 µg
1 x 400 µg = 400 µg
2900 µg

MALDI-TOF



Mixed 1.0 µl mycobactin sample at 1.0 mg/ml in $CHCl_3$ with 1.0 µl DHB matrix with 0.1% TFA, and analyzed in negative electrospray mode.

H-NMR



Ran 3.4 mg mycobactin suspended in CDCl3 on 400 MHz instrument, 128 scans in presaturated [H] mode. NMR indicates conversion of reactive hydroxyl groups to methoxy.

5/21/14
(Research Associate) date

5/23/14
(Laboratory Supervisor) date