

Mycobacterium tuberculosis*, Strain H37Rv, Mycobactin*Catalog No. NR-44101**

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Product Description:

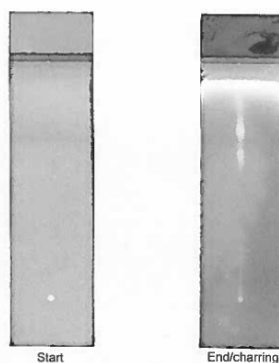
NR-44101 is a preparation of mycobactin derived from *Mycobacterium tuberculosis*, strain H37Rv.

Lot: 63449545¹**Manufacturing Date: 16NOV2015**

TEST	SPECIFICATIONS	RESULTS
Thin Layer Chromatography ²	Product visible after charring	Product visible after charring (Figure 1)
Identification by Mass Spectrometry ²	Report results	Pattern comparable to CSU-derived profile (Figure 2)

¹Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for bulk lot 15.Rv.11.13.1.MBT is attached.

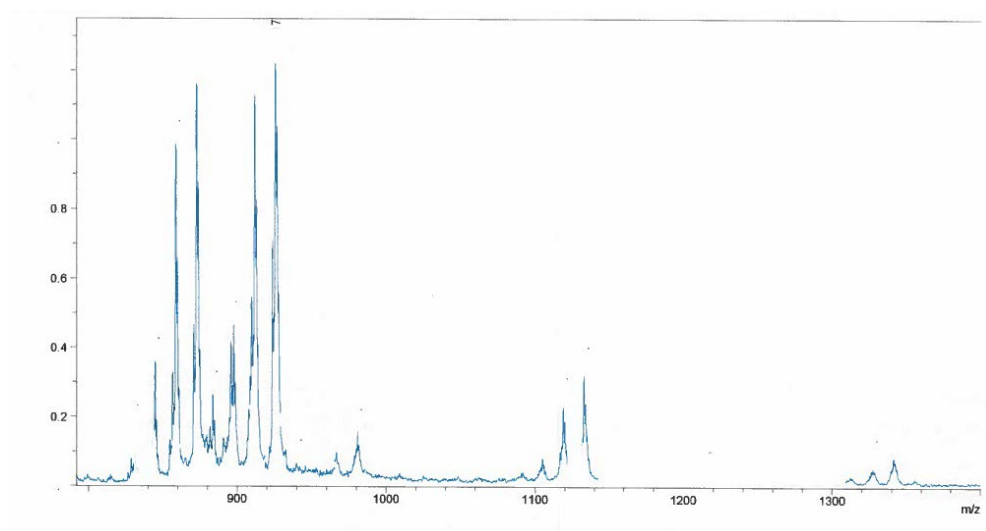
²Retesting of NR-44101 lot 63449545 was performed by BEI Resources in June 2019 to confirm product stability. One vial of NR-44101 lot 63449545 was suspended in 100 µL chloroform prior to testing.

Figure 1: Thin Layer Chromatography

NR-44101 lot 63449545 was resuspended in chloroform at a concentration of 1 µg/µL. ~ 10 µg of the sample was developed in 2/3/3 petroleum ether/n-butanol/ethyl acetate and visualized with copper sulfate and charring.

Figure 2: MALDI-TOF Mass Spectrometry of Mycobactin

The PGL-I spot from TLC was scraped and analyzed in positive electrospray mode with a dihydrobenzoic acid (DHB) matrix.



/Heather Couch/

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Program Manager or designee, ATCC Federal Solutions

05 SEP 2019

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected 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 MYCOBACTIN

General Information

BEI Catalog Number: NR-44101
CSU Lot Number: 15.Rv.11.13.1.MBT
Fraction Type: mycobactin
Species: *M. tuberculosis*
Strain: H37Rv

Purification Information

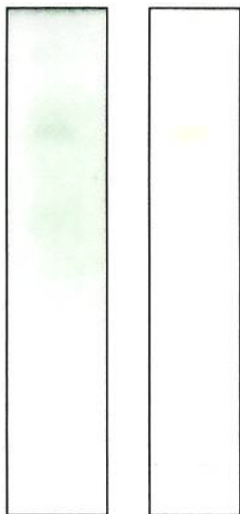
Starting material: 10:10:3 total lipid Starting Material Lot #: 13.Rv.2.7.23.6.WCg.a + b
Cells Irradiated: Yes Viability Test Performed: No Viable Organism Detected
Protocol used (SOP #'s): PP018.1, PP032.2, SP004, SP005, SP031b, SP032, SP033
Date started: 8/26/15
Date completed: 11/16/15
Notebook; page(s): Mycobactin Notebook 3 pp 1-33
Additional notes (if applicable): 10:10:3 total lipid was developed on preparative TLC plates with 95/5 chloroform/ methanol, and the visible mycobactin-bearing silica scraped. Crude mycobactin was extracted with 2:1 chloroform/ methanol. Subsequent clean-up was performed by developing crude extracts in preparative TLC plates with 2/3/3 petroleum ether/ n-butanol/ ethyl acetate.

Quality Control Information:

Total volume: 3.225 mL Total amount of mycobactin: 3.225 mg
Date dried on N₂ bath: 11/16/15
TLC date: 11/16/15 Notebook and page(s): Mycobactin 3 pp 33- 42
TLC Solvent System: 2/3/3 petroleum ether/ n-butanol/ ethyl acetate.

QC TLC:

CuSO₄ unstained

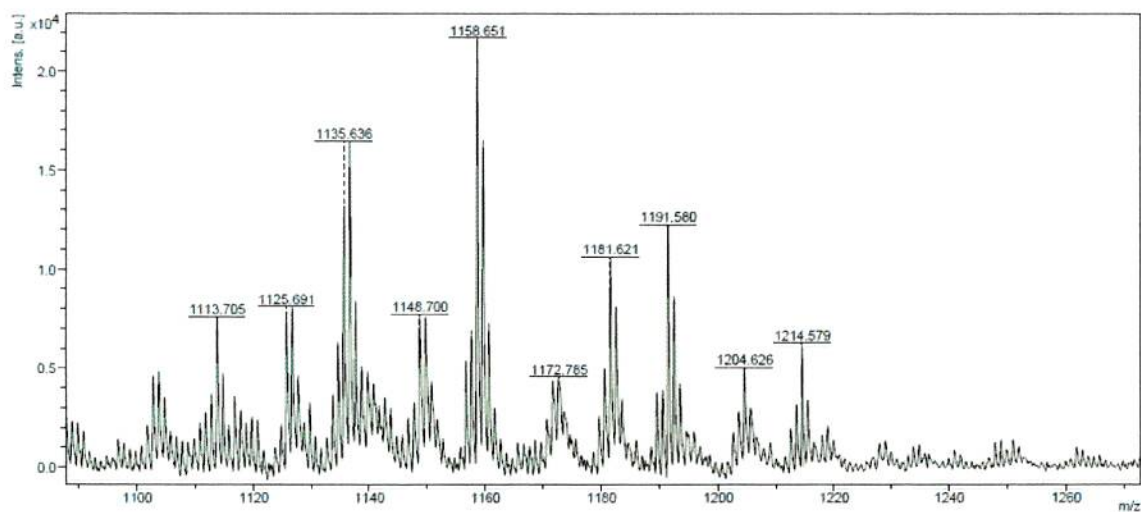
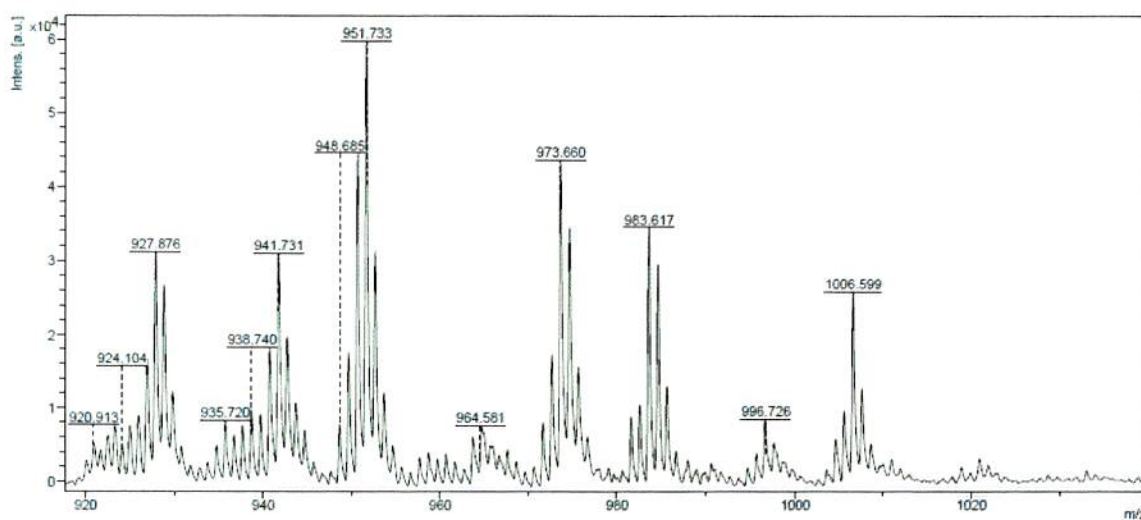
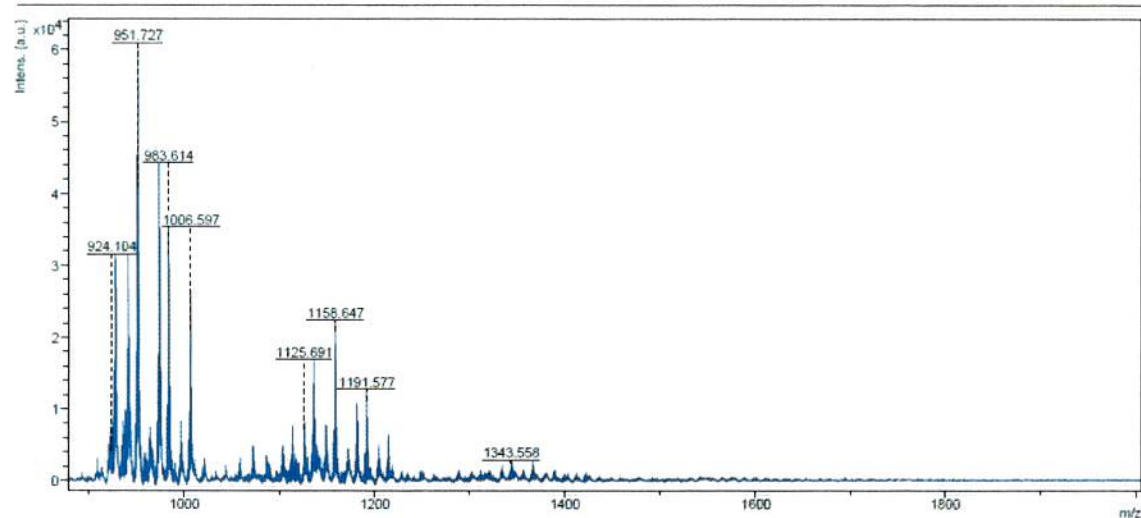


Aliquot Information:

20 x 100 ug = 2.0 mg
1 x 1225 ug = 1.225 mg
3.225 mg

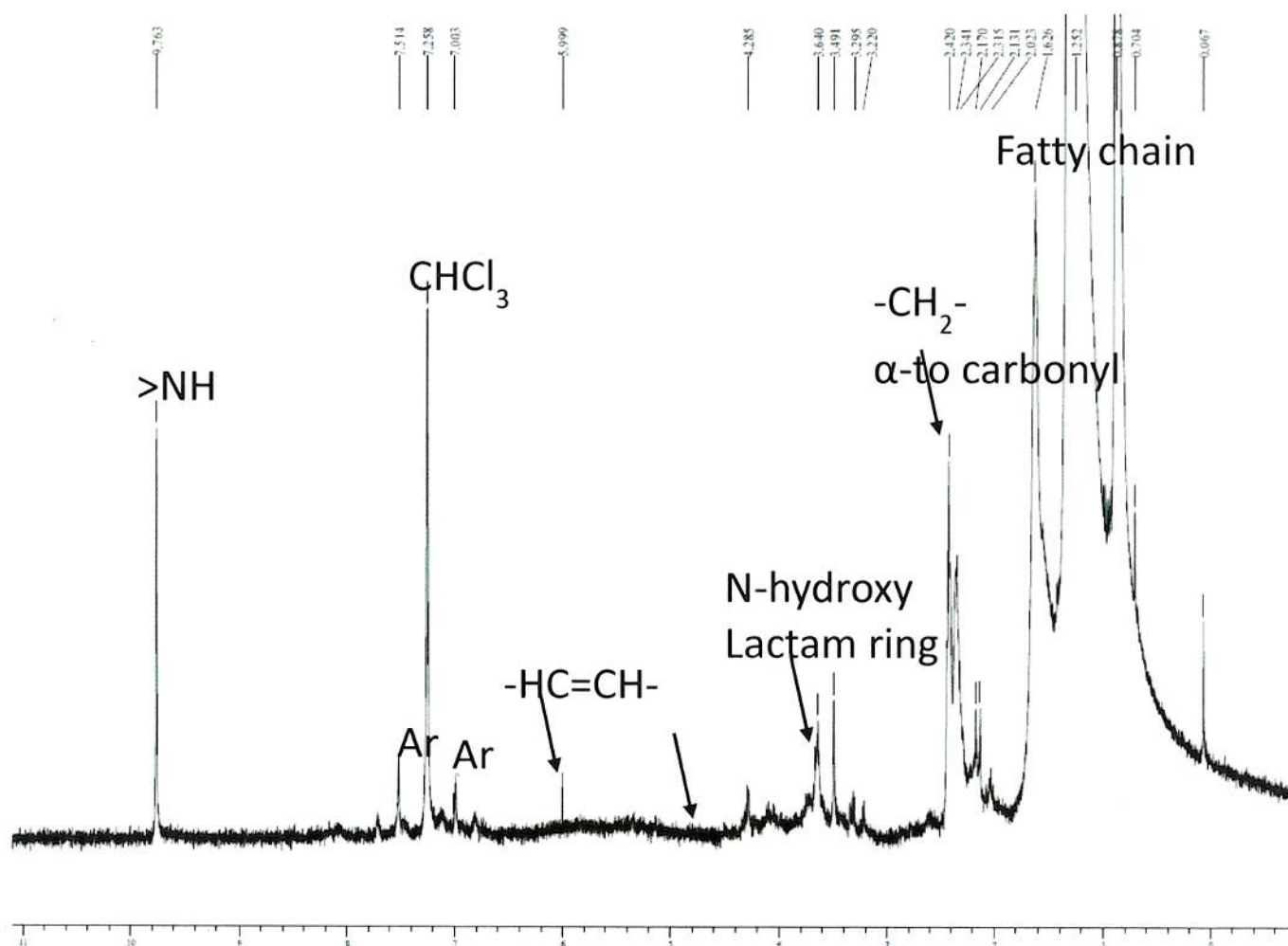
Loaded 50 ug, developed in solvent system described above, and stained one with CuSO₄ and charring.

MALDI-TOF:



Mixed 15.Rv.11.13.1.MBT at 1 ug/ul with DHB matrix 1:1 and analyzed in negative mode. Employed 80% intensity with method RN_PepMix.par.

H-NMR



Ran 3.3 mg mycobactin suspended in CDCl₃ on 400 MHz instrument, 128 scans in presaturated mode.

Sam L. J. Jansen 11/17/15
(Research Associate) date

C. McHaffey 11/18/15
(Laboratory Supervisor) date