

## Certificate of Analysis for NR-14847

# *Mycobacterium tuberculosis*, Strain H37Rv, Purified Phosphatidylinositol Mannoside 6 (PIM<sub>6</sub>)

Catalog No. NR-14847

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

**Product Description:** NR-14847 is a preparation of the purified phosphatidylinositol mannoside 6 (PIM<sub>6</sub>) cell wall glycolipids of irradiated *Mycobacterium tuberculosis*, strain H37Rv. The soluble organic fraction was extracted from irradiated cells, dried and titrated with cold acetone. The acetone-insoluble fraction was then applied to preparative thin-layer chromatography plates. PIMs were purified from the dried matrix using 40% methanol in chloroform.

Lot: 70005722 Manufacturing Date: 20JUN2017

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 17.Rv.6.20.JMR.PIM<sub>6</sub> is attached.

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#### WORK SHEET FOR PURIFIED PIM6

#### **General Information**

Lot Number: 17.Rv.6.20.JMR.PIM6 Species: Mycobacterium tuberculosis

Strain: H37Rv

#### **Purification Information**

Starting material (lot #): 14.Rv.2.6.24.5.WCA

Cell amount (wet weight): 72.9

Protocol used (SOP #): PP015.5, PP016.6, PP017.3

Date started: 02/09/2017 Date completed: 06/20/2017

Notebook; pages: <u>LLP#1 KN,JR,JB pgs 14,15,22,23,26-30</u>

Additional notes (if applicable): \_1 µg injected analyzed by LC-MS and spectra interrogated against the Mtb

LipidDB (Sartain et al, J Lipid Res 2011)

#### **Quality Control Information**

Clarity of product: Clear

Endotoxin amount: 0.52 ng/mg

GC Results: 0.58mg/ml

Total amount of PIM6: 2.95mg

Silver stain date: 06/02/17

Notebook and page(s): LLP#1 KN, JR, JB p30 Notebook and page(s): LLP#1 KN, JR, JB p29

Notebook and page(s): LLP#1 KN, JR, JB p30

#### QC Silver Stain and LC-MS:



Name	Notes	Mass	m/z	Quality Score
Ac1PIM2 (R1CO2H+R2CO2H+R3CO2H=51:0,R4=H)	[M-H]-	1414.908	1413.9	100
Ac1PIM6 (R1CO2H+R2CO2H+R3CO2H=51:0,R4=H)	[M-H]-	2063.122	1030.554	100
Ac2PIM2 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=67:0)	[M-H]-	1653.138	1652.13	87
Ac2PIM2 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=67:0)	[M-H]-	1653.139	1652.132	100
Ac2PIM2 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=69:0)	[M-H]-	1681.169	1680.162	100
Ac2PIM2 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=70:0)	[M-H]-	1695.186	1694.178	69.6
Ac2PIM2 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=70:0)	[M-H]-	1695.186	1694.179	100
Ac2PIM6 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=67:0)	[M+HAc- H]-	2361.369	1179.677	69.6
Ac2PIM6 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=67:0)	[M-H]-	2301.35	1149.668	100

	[M+Na-			
Ac2PIM6 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=67:0)	2H]-	2323.332	1160.659	100
Ac2PIM6 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=67:0)	[M+HAc- H]-	2361.369	1179.677	100
Ac2PIM6 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=69:0)	[M+HAc- H]-	2389.399	1193.691	73
Ac2PIM6 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=69:0)	[M-H]-	2329.38	1163.683	100
Ac2PIM6 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=69:1)	[M-H]-	2327.365	1162.676	100
Ac2PIM6 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=69:3)	[M+HAc- H]-	2383.352	1190.668	100
Ac2PIM6 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=70:0)	[M+HAc- H]-	2403.416	1200.7	69.6
Ac2PIM6 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=70:0)	[M-H]-	2343.397	1170.692	69.6
Ac2PIM6 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=70:0)	[M+Na- 2H]-	2365.378	1181.682	100
Ac2PIM6 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=70:0)	[M+HAc- H]-	2403.417	1200.701	100
Ac2PIM6 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=70:0)	[M-H]-	2343.398	1170.692	100
Ac2PIM6 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=72:0)	[M-H]-	2371.429	1184.707	100
Ac2PIM6 (R1CO2H+R2CO2H+R3CO2H+R4CO2H=72:3)	[M+HAc- H]-	2425.398	1211.693	100
DG (R1CO2H+R2CO2H=34:0)	[M+HAc- H]-	656.5621	655.5548	55.7
DG (R1CO2H+R2CO2H=36:0)	[M+HAc- H]-	684.5933	683.5861	67.5
DG (R1CO2H+R2CO2H=36:0)	[M+HAc- H]-	684.5937	683.5863	86.3
Mycocerosic acid (C35)	[M+HAc- H]-	582.5612	641.5756	100
PI (R1CO2H+R2CO2H=33:0)	[M+HAc- H]-	884.5693	929.5648	67.3
PI (R1CO2H+R2CO2H=36:0)	[M-H]-	866.5903	847.5726	81.1
PI (R1CO2H+R2CO2H=36:0)	[M-H]-	866.5903	847.5728	100

### \*Trace PIM2 detected in sample

Aliquot Information:

11 vials @ 250μg 1 vial@200ug retained by CSU