

Certificate of Analysis for NR-18999

Mycobacterium tuberculosis, Strain HN2304

Catalog No. NR-18999

Product Description: *Mycobacterium tuberculosis* (*M. tuberculosis*), strain HN2304 was isolated in 2000 from human pulmonary tissue in Texas, USA. Strain HN2304 was deposited as a non-drug resistant strain.

Lot¹: 63344537 Manufacturing Date: 20MAR2015

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ²		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ³	Report results	Irregular, slight peaked, undulate, rough, opaque and cream
		(Figure 1)
Growth rate	≥ 7 days	30 days
Growth at 26°C	Negative	Negative
Growth at 37°C	Positive	Positive
Acid-fast stain	Positive (red colonies)	Positive (red colonies)
Pigmentation in the dark (Scotochromogen)	Negative (no pigment)	Negative (no pigment)
Photoinduction for 1 hour (Photochromogen)	Negative (no pigment)	Negative (no pigment)
Nonchromogen (no pigment)	Positive (no pigment)	Positive (no pigment)
Biochemical tests		
Niacin production ⁴	Positive	Positive
Nitrate reduction	Positive	Positive
Pyrazinamidase	Positive	Positive
Antibiotic Susceptibility Profile		
Sensititre™ System ^{5,6}		
Amikacin	Report results	≤ 0.12 μg/mL
Cycloserine	Report results	= 4 µg/mL
Ethambutol	Report results	≤ 0.5 µg/mL
Ethionamide	Report results	= 1.2 µg/mL
Isoniazid	Report results	≤ 0.03 µg/mL
Kanamycin	Report results	≤ 0.6 µg/mL
Moxifloxacin	Report results	= 0.12 µg/mL
Ofloxacin	Report results	≤ 0.25 μg/mL
Para-aminosalicylic acid	Report results	≤ 0.5 µg/mL
Rifabutin	Report results	≤ 0.12 µg/mL
Rifampin	Report results	≤ 0.12 μg/mL
Streptomycin	Report results	≤ 0.25 µg/mL
Genotypic Analysis	≥ 99% sequence identity to	100% sequence identity to
Sequencing of Heat Shock Protein 65 gene	M. tuberculosis type strain	M. tuberculosis type strain
(~ 430 base pairs)	(GenBank: AL123456)	(GenBank: AL123456) ⁷
Purity (post-freeze) ⁸	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze) ³	Growth	Growth
		<u> </u>

¹NR-18999 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment. Broth inoculum was added to Middlebrook 7H10 agar with OADC enrichment kolles, which were grown for 23 days at 37°C in an aerobic atmosphere with 5% CO₂ to produce this lot.

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Information on Mycobacterium testing is available from Ribón, W. "Biochemical Isolation and Identification of Mycobacteria" <u>Biochemical Testing</u>. (2012) Jose C. Jimenez-Lopez (Ed.), InTech, http://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacteria and Lévy-Frébault, V. V. and F. Portaels. "Proposed Minimal Standards for the Genus *Mycobacterium* and for Description of New Slowly Growing *Mycobacterium* Species." <a href="https://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacterial and Lévy-Frébault, V. V. and F. Portaels. "Proposed Minimal Standards for the Genus *Mycobacterium* and for Description of New Slowly Growing *Mycobacterium* Species." https://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacteria

³30 days at 37°C in an aerobic atmosphere with 5% CO₂ on Middlebrook 7H10 agar with OADC enrichment

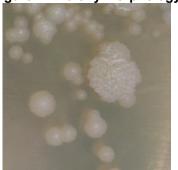
⁴All mycobacteria produce niacin but only *M. tuberculosis* accumulates it, resulting in a positive test for *M. tuberculosis*.



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⁵Sensititre[™] System *Mycobacterium tuberculosis* MIC Plate, Thermo Scientific[™], catalog number MYCOTB

Figure 1: Colony Morphology



Signature: **Date: 15 NOV 2016**

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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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 $^{^6}$ No interpretations of the Sensititre[™] System data for *M. tuberculosis* are currently available. 7 Also consistent with *M. africanum*, *M. bovis* and *M. microti*

⁸Purity of this lot was assessed for 40 days at 37°C in an aerobic atmosphere with 5% CO₂ on Middlebrook 7H10 agar with OADC enrichment and on Tryptic Soy agar plates.