

Certificate of Analysis for NR-30936

Mycobacterium tuberculosis, Strain 96-3297

Catalog No. NR-30936

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

Product Description: *Mycobacterium tuberculosis (M. tuberculosis)*, strain 96-3297 was isolated between 1995 and 2000 from human sputum from an HIV-positive patient infected with pulmonary tuberculosis in North America.

Lot¹: 70005379 Manufacturing Date: 07JUL2017

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ²		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ³	Report results	Irregular, slight peaked, undulate,
Total mark margy		rough and cream (Figure 1)
Growth rate	≥ 7 days	21 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	0.5 μg/mL ^{7,8}
Cycloserine	Report results	32 µg/mL ^{8,9}
Ethambutol	Report results	1 μg/mL ¹⁰
Ethionamide	Report results	0.6 μg/mL ¹⁰
Isoniazid	Report results	≤ 0.03 μg/mL
Kanamycin	Report results	2.5 μg/mL
Moxifloxacin	Report results	0.12 µg/mL
Ofloxacin	Report results	1 μg/mL ^{8,11}
Para-aminosalicylic acid	Report results	≤ 0.5 μg/mL ¹⁰
Rifabutin	Report results	≤ 0.12 µg/mL ¹⁰
Rifampin	Report results	≤ 0.12 µg/mL
Streptomycin	Report results	0.5 μg/mL ^{8,10,12}
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene	≥ 99% sequence identity to	100% sequence identity to
(~ 420 base pairs)	M. tuberculosis type strain	M. tuberculosis type strain
(.25 2300 pano)	(GenBank: AL123456)	(GenBank: AL123456) ¹³
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment ¹⁴	Growth consistent with expected	Growth consistent with expected
	colony morphology	colony morphology
Tryptic Soy agar ¹⁵	Report results	Growth consistent with expected
Tryphic Goy agai	Report results	colony morphology
		Colony morphology
Viability (post-freeze) ³	Growth	Growth
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¹NR-30936 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 32 days at 37°C in an aerobic atmosphere with 5% CO₂ to produce this lot.

BEI Resources

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²Information 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." <u>Int. J. Syst. Bacteriol.</u> 42 (1992): 315-323. PubMed: 1581193.

³21 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*.

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

⁶Minimum Inhibitory Concentration (MIC); No Clinical & Laboratory Standards Institute (CLSI) interpretations of the Sensititre™ System data for *M. tuberculosis* are currently available.

⁷Two MICs were observed for amikacin (0.25 μg/mL and 0.5 μg/mL) under identical test conditions. The highest MIC is being reported as the test result.

⁸Variability in the MIC result by the Sensititre[™] method has been demonstrated (Lee, J., et al. "Sensititre MYCOTB MIC Plate for Testing *Mycobacterium tuberculosis* Susceptibility to First- and Second-Line Drugs." <u>Antimicrob. Agents Chemother.</u> 58 (2014): 11-18. PubMed: 24100497.), with the results for a single antibiotic typically within one doubling dilution.

⁹Two MICs were observed for cycloserine (16 μg/mL and 32 μg/mL) under identical test conditions. The highest MIC is being reported as the test result.

¹⁰For streptomycin, ethionamide, para-aminosalicylic acid, rifabutin and ethambutol, the endpoint for these drugs is determined by the well with approximately 80% inhibition of growth compared to the positive control well with no drug.

11Two MICs were observed for ofloxacin (0.5 µg/mL and 1 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.

12Two MICs were observed for streptomycin (≤ 0.25 μg/mL and 0.5 μg/mL) under identical test conditions. The highest MIC is being reported as the test result.

¹³Also consistent with *M. africanum*, *M. bovis*, *M. canettii*, *M. caprae* and *M. microti*

¹⁴Purity of this lot was assessed for 59 days at 37°C in an aerobic atmosphere with 5% CO₂.

¹⁵Purity of this lot was assessed for 21 days at 37°C in an aerobic atmosphere with 5% CO₂.

Figure 1: Colony Morphology



/Heather Couch/ Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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