

Certificate of Analysis for NR-30925

Mycobacterium tuberculosis, Strain 96-2199

Catalog No. NR-30925

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

Product Description: *Mycobacterium tuberculosis (M. tuberculosis)*, strain 96-2199 was isolated between 1995 and 2000 from human sputum from an HIV-positive patient infected with pulmonary tuberculosis in North America.

Lot¹: 70004765 Manufacturing Date: 01JUN2017

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ²		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ³	Report results	Circular, raised, entire, rough and
Total mark margy		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		(1 5 /
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
Cycloserine	Report results	16 µg/mL
Ethambutol	Report results	≤ 0.5 μg/mL ⁸
Ethionamide	Report results	0.6 µg/mL ⁸
Isoniazid	Report results	0.06 µg/mL
Kanamycin	Report results	1.2 µg/mL
Moxifloxacin	Report results	0.25 μg/mL
Ofloxacin	Report results	0.5 µg/mL ⁹
Para-aminosalicylic acid	Report results	≤ 0.5 μg/mL ⁸
Rifabutin	Report results	≤ 0.12 µg/mL ⁸
Rifampin	Report results	≤ 0.12 µg/mL
Streptomycin	Report results	≤ 0.25 µg/mL ⁸
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene	≥ 99% sequence identity to	99% sequence identity to
(~ 410 base pairs)	M. tuberculosis type strain	M. tuberculosis type strain
(~ 410 base pails)	(GenBank: AL123456)	(GenBank: AL123456) ¹⁰
	(Genbank, AL 125450)	(Gendank, AL123430)
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment ¹¹	Growth consistent with expected	Growth consistent with expected
	colony morphology	colony morphology
Tryptic Soy agar ¹²	Report results	Growth consistent with expected
		colony morphology ¹³
Viability (post-freeze) ³	Growth	Growth
	0.0701	0.0.701

¹NR-30925 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 21 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." Int. J. Syst. Bacteriol. 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. Variability 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." Antimicrob. Agents Chemother. 58 (2014): 11-18. PubMed: 24100497), with the results for a single antibiotic typically within one doubling dilution.

⁸For 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.

⁹Two MICs were observed for ofloxacin (≤ 0.25 μg/mL and 0.5 μg/mL) under identical test conditions. The highest MIC is being reported as the test result. Variability 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." Antimicrob. Agents Chemother. 58 (2014): 11-18. PubMed: 24100497), with the results for a single antibiotic typically within one doubling dilution.

¹⁰Also consistent with M. africanum, M. bovis, M. canettii, M. caprae and M. microti

¹¹Purity of this lot was assessed for 35 days at 37°C in an aerobic atmosphere with 5% CO₂.

¹²Purity of this lot was assessed for 21 days at 37°C in an aerobic atmosphere with 5% CO₂.

¹³A small number of tiny colonies were observed on the primary inoculation zone as a result of residual growth medium present in the inoculate.



Figure 1: Colony Morphology

/Heather Couch/ Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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