

Certificate of Analysis for NR-30921

Mycobacterium tuberculosis, Strain 95-2477

Catalog No. NR-30921

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

Product Description: *Mycobacterium tuberculosis (M. tuberculosis)*, strain 95-2477 was isolated between 1995 and 2000 from human sputum from an HIV-positive patient infected with pulmonary tuberculosis in North America.

Lot¹: 70004756 Manufacturing Date: 31MAY2017

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ²		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ³	Report results	Circular, raised, entire, 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	0.25 μg/mL
	Report results	32 μg/mL
Cycloserine Ethambutol	Report results	32 μg/mL ⁷ ≤ 0.5 μg/mL ⁷
Ethambuloi Ethionamide	Report results	0.6 μg/mL ⁷
		0.6 μg/mL ≤ 0.03 μg/mL
Isoniazid	Report results Report results	2.5 μg/mL
Kanamycin Moxifloxacin	Report results	2.5 μg/mL ⁸
		1 μg/mL ⁹
Ofloxacin	Report results Report results	1 μg/mL ³ ≤ 0.5 μg/mL ⁷
Para-aminosalicylic acid		$\leq 0.12 \mu \text{g/mL}^7$
Rifabutin	Report results	
Rifampin	Report results Report results	≤ $0.12 \mu g/mL$ ≤ $0.25 \mu g/mL^7$
Streptomycin	Report results	≤ 0.25 μg/IIIL
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
	(GenBank: AL123456)	(GenBank: AL123456)10
Purity (post froozo)		
Purity (post-freeze)	Crowth consistent with over seter	Crouth consistant with over seted
Middlebrook 7H10 agar with OADC enrichment ¹¹	Growth consistent with expected	Growth consistent with expected
Tryptic Soy agar ¹²	colony morphology Report results	colony morphology Growth consistent with expected
Trypuo soy agar-	Vehou iezniiz	colony morphology ¹³
		colony morphology.
Viability (post-freeze) ³	Growth	Growth
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¹NR-30921 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.

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.

⁷For 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.

⁸Two MICs were observed for moxifloxacin (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.

⁹Two 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. 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." <u>Antimicrob. Agents Chemother.</u> 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.

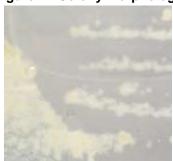


Figure 1: Colony Morphology

/Heather Couch/ Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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