

Certificate of Analysis for NR-30914

Mycobacterium tuberculosis, Strain 99-2165

Catalog No. NR-30914

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

Product Description: *Mycobacterium tuberculosis (M. tuberculosis)*, strain 99-2165 was isolated between 1995 and 2000 from human sputum from an HIV-negative patient infected with pulmonary tuberculosis in North America.

Lot¹: 70004742 Manufacturing Date: 30MAY2017

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ²		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ³	Report results	Irregular, slight peaked, undulate,
		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
Cycloserine	Report results	16 µg/mL
Ethambutol	Report results	2 μg/mL ^{7,8}
Ethionamide	Report results	1.2 µg/mL ⁷
Isoniazid	Report results	≤ 0.03 µg/mL
Kanamycin	Report results	2.5 μg/mL
Moxifloxacin	Report results	0.25 μg/mL
Ofloxacin	Report results	1 μg/mL
Para-aminosalicylic acid	Report results	$\leq 0.5 \mu \text{g/mL}^7$
Rifabutin	Report results	$\leq 0.12 \mu \text{g/mL}^7$
Rifampin	Report results	≤ 0.12 μg/mL
Streptomycin	Report results	0.5 μg/mL ⁷
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene	≥ 99% sequence identity to	100% sequence identity to
(~ 380 base pairs)	M. tuberculosis type strain	M. tuberculosis type strain
· ,	(GenBank: AL123456)	(GenBank: AL123456) ⁹
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment ¹⁰	Growth consistent with expected	Growth consistent with expected
	colony morphology	colony morphology
Tryptic Soy agar ¹¹	Report results	Growth consistent with expected
		colony morphology
Viability (post-freeze) ³	Growth	Growth

NR-30914 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 25 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-ofmycobacteria 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 tuberculosi*s 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.

For 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 ethambutol (1 μg/mL and 2 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 Second-Line tuberculosis Susceptibility to Firstand Drugs." Agents Chemother. Antimicrob. (2014): 11-18. PubMed: 24100497.), with the results for a single antibiotic typically within one doubling dilution.

⁹Also consistent with *M. africanum, M. bovis, M. canettii, M. caprae* and *M. microti*

¹⁰Purity of this lot was assessed for 52 days at 37°C in an aerobic atmosphere with 5% CO₂.

¹¹Purity of this lot was assessed for 21 days at 37°C in an aerobic atmosphere with 5% CO₂.





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Program Manager or designee, ATCC Federal Solutions

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