

Certificate of Analysis for NR-30858

Mycobacterium tuberculosis, Strain 98-2462

Catalog No. NR-30858

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

Product Description: *Mycobacterium tuberculosis (M. tuberculosis)*, strain 98-2462 was isolated between 1995 and 2000 from human sputum from an HIV-negative patient infected with pulmonary tuberculosis in North America.

Lot¹: 70002568 Manufacturing Date: 16MAY2017

| TEST | SPECIFICATIONS | RESULTS |
|--|---------------------------------|---|
| Phenotypic Analysis ² | | |
| Cellular morphology | Gram-positive rods | Gram-positive rods |
| Colony morphology ³ | Report results | Irregular, raised, undulate, rough and cream (Figure 1) |
| Growth rate | ≥ 7 days | 37 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 | . semire (ine piginemy | l cours (no pigmon) |
| 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 | 8 μg/mL |
| Ethambutol | Report results | $\leq 0.5 \mu \text{g/mL}^7$ |
| Ethionamide | Report results | ≤ 0.3 μg/mL ⁷ |
| Isoniazid | Report results | ≤ 0.03 µg/mL |
| Kanamycin | Report results | 1.2 µg/mL |
| Moxifloxacin | Report results | 0.25 µg/mL |
| Ofloxacin | Report results | 1 μg/mL ⁸ |
| Para-aminosalicylic acid | Report results | $\leq 0.5 \mu \text{g/mL}^7$ |
| Rifabutin | Report results | ≤ 0.12 /mL ⁷ |
| Rifampin | Report results | ≤ 0.12 µg/mL |
| Streptomycin | Report results | ≤ 0.25 µg/mL ⁷ |
| Genotypic Analysis | | |
| Sequencing of Heat Shock Protein 65 gene | ≥ 99% sequence identity to | 100% sequence identity to |
| (~ 370 base pairs) | M. tuberculosis type strain | M. tuberculosis type strain |
| (5: 5: 5: 5: 5: 5: 5: 5: 5: 5: 5: 5: 5: | (GenBank: AL123456) | (GenBank: AL123456) ⁹ |
| Purity (post-freeze) | | |
| Middlebrook 7H10 agar with OADC enrichment ¹⁰ | Growth consistent with expected | Growth consistent with expected |
| G | colony morphology | colony morphology |
| Tryptic Soy agar ¹⁰ | Report results | Growth consistent with expected |
| | | colony morphology ¹¹ |
| Viability (post-freeze) ³ | Growth | Growth |
| | 1 | I . |

¹NR-30858 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 55 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." https://example.com/intention-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." https://example.com/intention-and-identification-of-mycobacterium 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://example.com/intention-and-identification-of-mycobacterium and Lévy-Frébault, V. V. and F. Portaels. "Proposed Minimal Standards for the Genus *Mycobacterium* and for Description of New Slowly Growing Mycobacterium" (Addition-and-identification-of-mycobacterium) (Addi

37 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 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 oflaxacin (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." Antimicrob. Agents Chemother. 58 (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 37 days at 37°C in an aerobic atmosphere with 5% CO₂.

¹¹A 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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