

Certificate of Analysis for NR-43805

Mycobacterium tuberculosis, Strain KT-0020

Catalog No. NR-43805

Product Description:

Mycobacterium tuberculosis (M. tuberculosis), strain KT-0020 was isolated from a human in South Korea. Strain KT-0020 was deposited as a Beijing genotype strain with resistance to rifampin. NR-43805 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 38 days at 37°C in an aerobic atmosphere with 5% CO₂ to produce this lot.

Lot: 70014689 Manufacturing Date: 01JUN2018

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TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ¹		
Cellular morphology	Gram-positive rods	Gram-positive rods
21 days at 37°C in an aerobic atmosphere with 5% CO ₂		
on Middlebrook 7H10 agar with OADC enrichment		
Colony morphology	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 ²	Positive	Positive
Nitrate reduction	Positive	Positive
Antibiotic Susceptibility Profile		
Sensititre™ System ^{3,4}		
Amikacin	Report results	0.25 μg/mL
Cycloserine	Report results	64 μg/mL ^{5,6,7}
Ethambutol	Report results	1 μg/mL ⁵
Ethionamide	Report results	1.2 µg/mL ^{5,7,8}
Isoniazid	Report results	0.06 μg/mL ^{7,9}
Kanamycin	Report results	2.5 μg/mL
Moxifloxacin	Report results	1 μg/mL
Ofloxacin	Report results	2 μg/mL ^{7,10}
Para-aminosalicylic acid	Report results	64 μg/mL ⁵
Rifabutin	Report results	1 μg/mL ⁵
Rifampin	Report results	> 16 µg/mL
Streptomycin	Report results	0.5 μg/mL ⁵
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene (~ 1620 base pairs)	≥ 99% sequence identity to <i>M. tuberculosis</i> , strain KT-0020 (GenBank: JLNP01000004.1)	100% sequence identity to M. tuberculosis, strain KT-0020 (GenBank: JLNP01000004.1) ¹¹
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment 46 days at 37°C in an aerobic atmosphere with 5% CO ₂	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology

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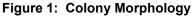


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TEST	SPECIFICATIONS	RESULTS
Purity (post-freeze)		
Tryptic Soy agar	Report results	Growth consistent with expected
21 days at 37°C in an aerobic atmosphere with 5% CO ₂		colony morphology
Viability (post-freeze)	Growth	Growth
21 days at 37°C in an aerobic atmosphere with 5% CO ₂		
on Middlebrook 7H10 agar with OADC enrichment		

¹Information 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/intentification-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/intentification-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/intentification-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/intentification-of-mycobacteria and Lévy-Frébault, V. V. and F. Portaels. "Proposed Minimal Standards for the Genus Mycobacterium and for Description of New Slowly Standards for the Mycobacteria" of New Slowly Standards for the Mycobacteria and Mycobac

¹¹Also consistent with M. africanum, M. bovis, M. canettii, M. caprae and M. microti





/Heather Couch/ Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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Tel: 800-359-7370

Fax: 703-365-2898

²All mycobacteria produce niacin but only *M. tuberculosis* accumulates it, resulting in a positive test for *M. tuberculosis*.

³Sensititre™ System Mycobacterium tuberculosis MIC Plate, Thermo Scientific™, catalog number MYCOTB

⁴Minimum Inhibitory Concentration (MIC); No Clinical & Laboratory Standards Institute (CLSI) interpretations of the Sensititre™ System data for *M. tuberculosis* are currently available.

⁵For ethambutol, ethionamide, para-aminosalicylic acid, rifabutin and streptomycin, 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.

⁶Two MICs were observed for cycloserine (32 µg/mL and 64 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.

⁷Variability 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.

⁸Two MICs were observed for ethionamide (0.6 μg/mL and 1.2 μg/mL) under identical test conditions. The highest MIC is being reported as the test result.

⁹Two MICs were observed for isoniazid (≤ 0.03 μg/mL and 0.06 μg/mL) under identical test conditions. The highest MIC is being reported as the test result.

¹⁰Two MICs were observed for ofloxacin (1 μg/mL and 2 μg/mL) under identical test conditions. The highest MIC is being reported as the test result.