

# **Certificate of Analysis for NR-43794**

### Mycobacterium tuberculosis, Strain KT-0009

#### Catalog No. NR-43794

#### **Product Description:**

Mycobacterium tuberculosis (M. tuberculosis), strain KT-0009 was isolated from a human in South Korea. Strain KT-0009 was deposited as an extensively drug-resistant (XDR) Beijing genotype strain with resistance to isoniazid, kanamycin, moxifloxacin, ofloxacin and rifampin. NR-43794 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 44 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub> to produce this lot.

Lot: 70013979 Manufacturing Date: 01JUN2018

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis <sup>1</sup>		
Cellular morphology	Gram-positive rods	Gram-positive rods
21 days at 37°C in an aerobic atmosphere with		
5% CO <sub>2</sub> on Middlebrook 7H10 agar with OADC		
enrichment	Donort coults	
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 <sup>2</sup>	Positive	Positive
Nitrate reduction	Positive	Positive
Antibiotic Susceptibility Profile		
Sensititre™ System <sup>3,4</sup>		
Amikacin	Report results	0.5 μg/mL
Cycloserine	Report results	32 μg/mL <sup>5,6</sup>
Ethambutol	Report results	4 μg/mL <sup>7</sup>
Ethionamide	Report results	5 μg/mL <sup>6,7,8</sup>
Isoniazid	Report results	> 4 µg/mL
Kanamycin	Report results	20 μg/mL <sup>6,9</sup>
Moxifloxacin	Report results	8 μg/mL <sup>6,10</sup>
Ofloxacin	Report results	16 μg/mL <sup>6,11</sup>
Para-aminosalicylic acid	Report results	1 μg/mL <sup>7</sup>
Rifabutin	Report results	1 μg/mL <sup>6,7,12</sup>
Rifampin	Report results	> 16 µg/mL
Streptomycin	Report results	0.5 μg/mL <sup>7</sup>
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene	≥ 99% sequence identity to	100% sequence identity to
(~ 1620 base pairs)	M. tuberculosis type strain	M. tuberculosis type strain
	(GenBank: AL123456.3)	(GenBank: AL123456.3) <sup>13</sup>
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment	Growth consistent with expected	Growth consistent with expected
31 days at 37°C in an aerobic atmosphere with 5% CO <sub>2</sub>	colony morphology	colony morphology
Tryptic Soy agar	Report results	Growth consistent with expected
21 days at 37°C in an aerobic atmosphere with 5% CO <sub>2</sub>		colony morphology

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org Tel: 800-359-7370

Fax: 703-365-2898



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TEST	SPECIFICATIONS	RESULTS
Viability (post-freeze) 21 days at 37°C in an aerobic atmosphere with 5% CO <sub>2</sub>	Growth	Growth
on Middlebrook 7H10 agar with OADC enrichment		

Information 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, <a href="http://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacteria">http://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacteria</a> and Lévy-Frébault, V. V. and F. Portaels. "Proposed Minimal Standards for the Genus Mycobacterium and for Description of New Slowly Growing Mycobacterium Species." <a href="https://link.doi.org/10.1008/jns.

Figure 1: Colony Morphology



/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

<sup>&</sup>lt;sup>2</sup>All 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

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

<sup>&</sup>lt;sup>5</sup>Two MICs were observed for cycloserine (16 μg/mL and 32 μg/mL) under identical test conditions. The highest MIC is being reported as the test result. <sup>6</sup>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.

<sup>&</sup>lt;sup>7</sup>For 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.

<sup>&</sup>lt;sup>8</sup>Two MICs were observed for ethionamide (2.5 μg/mL and 5 μg/mL) under identical test conditions. The highest MIC is being reported as the test result.

<sup>9</sup>Two MICs were observed for kanamycin (10 μg/mL and 20 μg/mL) under identical test conditions. The highest MIC is being reported as the test result.

<sup>10</sup>Two MICs were observed for moxifloxacin (4 μg/mL and 8 μg/mL) under identical test conditions. The highest MIC is being reported as the test result.

<sup>&</sup>lt;sup>11</sup>Two MICs were observed for ofloxacin (8 µg/mL and 16 µg/mL) under identical test conditions. The highest MIC is being reported as the test result. <sup>12</sup>Two MICs were observed for rifabutin (0.5 µg/mL and 1 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.

<sup>&</sup>lt;sup>13</sup>Also consistent with *M. africanum*, *M. bovis*, *M. canettii*, *M. caprae* and *M. microti*