

***Mycobacterium tuberculosis*, Strain 99-2424**

**Catalog No. NR-30918**

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**Product Description:** *Mycobacterium tuberculosis* (*M. tuberculosis*), strain 99-2424 was isolated between 1995 and 2000 from human sputum from an HIV-negative patient infected with pulmonary tuberculosis in North America.

**Lot<sup>1</sup>: 70004750**

**Manufacturing Date: 29JUN2017**

TEST	SPECIFICATIONS	RESULTS
<b>Phenotypic Analysis<sup>2</sup></b> Cellular morphology Colony morphology <sup>3</sup>  Growth rate Growth at 26°C Growth at 37°C Acid-fast stain Pigmentation in the dark (Scotochromogen) Photoinduction for 1 hour (Photochromogen) Nonchromogen (no pigment) Biochemical tests Niacin production <sup>4</sup> Nitrate reduction Pyrazinamidase	Gram-positive rods Report results  ≥ 7 days Negative Positive Positive (red colonies) Negative (no pigment) Negative (no pigment) Positive (no pigment)  Positive Positive Positive	Gram-positive rods Irregular, slight peaked, undulate, rough and cream (Figure 1) 21 days Negative Positive Positive (red colonies) Negative (no pigment) Negative (no pigment) Negative (no pigment) Positive (no pigment)  Positive Positive Positive
<b>Antibiotic Susceptibility Profile</b> Sensititre™ System <sup>5,6</sup> Amikacin Cycloserine Ethambutol Ethionamide Isoniazid Kanamycin Moxifloxacin Ofloxacin Para-aminosalicylic acid Rifabutin Rifampin Streptomycin	Report results Report results Report results Report results Report results Report results Report results Report results Report results Report results Report results Report results Report results Report results	0.25 µg/mL 16 µg/mL 1 µg/mL <sup>7</sup> 2.5 µg/mL <sup>7,8,9</sup> 0.06 µg/mL <sup>9,10</sup> 1.2 µg/mL 0.5 µg/mL <sup>9,11</sup> 1 µg/mL 1 µg/mL <sup>7</sup> 0.25 µg/mL <sup>7,9,12</sup> 0.25 µg/mL 2 µg/mL <sup>7,9,13</sup>
<b>Genotypic Analysis</b> Sequencing of Heat Shock Protein 65 gene (~ 410 base pairs)	≥ 99% sequence identity to <i>M. tuberculosis</i> type strain (GenBank: AL123456)	100% sequence identity to <i>M. tuberculosis</i> type strain (GenBank: AL123456) <sup>14</sup>
<b>Purity (post-freeze)</b> Middlebrook 7H10 agar with OADC enrichment <sup>15</sup>  Tryptic Soy agar <sup>16</sup>	Growth consistent with expected colony morphology Report results	Growth consistent with expected colony morphology Growth consistent with expected colony morphology
<b>Viability (post-freeze)<sup>3</sup></b>	Growth	Growth

<sup>1</sup>NR-30918 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment and grown for 23 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub>. Broth inoculum was added to Middlebrook 7H10 agar with OADC enrichment kolles, which were grown for 29 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub> to produce this lot.

- <sup>2</sup>Information on Mycobacterium testing is available from Ribón, W. "Biochemical Isolation and Identification of Mycobacteria." Biochemical Testing. (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.
- <sup>3</sup>21 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub> on Middlebrook 7H10 agar with OADC enrichment
- <sup>4</sup>All mycobacteria produce niacin but only *M. tuberculosis* accumulates it, resulting in a positive test for *M. tuberculosis*.
- <sup>5</sup>Sensititre™ System *Mycobacterium tuberculosis* MIC Plate, Thermo Scientific™, catalog number MYCOTB
- <sup>6</sup>Minimum Inhibitory Concentration (MIC); No Clinical & Laboratory Standards Institute (CLSI) interpretations of the Sensititre™ System data for *M. tuberculosis* are currently available.
- <sup>7</sup>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.
- <sup>8</sup>Two MICs were observed for ethionamide (1.2 µg/mL and 2.5 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.
- <sup>9</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." Antimicrob. Agents Chemother. 58 (2014): 11-18. PubMed: 24100497.), with the results for a single antibiotic typically within one doubling dilution.
- <sup>10</sup>Two 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.
- <sup>11</sup>Two 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.
- <sup>12</sup>Two MICs were observed for rifabutin (≤ 0.12 µg/mL and 0.25 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.
- <sup>13</sup>Two MICs were observed for streptomycin (1 µg/mL and 2 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.
- <sup>14</sup>Also consistent with *M. africanum*, *M. bovis*, *M. canettii*, *M. caprae* and *M. microti*
- <sup>15</sup>Purity of this lot was assessed for 52 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub>.
- <sup>16</sup>Purity of this lot was assessed for 21 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub>.

Figure 1: Colony Morphology



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