

***Mycobacterium tuberculosis*, Strain XTB13-108**

**Catalog No. NR-49365**

**Product Description:** *Mycobacterium tuberculosis* (*M. tuberculosis*), strain XTB13-108 was isolated in 2012 from the sputum of a patient with tuberculosis in the Republic of Belarus. Strain XTB13-108 was deposited as resistant to amikacin, capreomycin, ethambutol, isoniazid, kanamycin, ofloxacin, rifampin and streptomycin.

**Lot<sup>1</sup>: 64064218**

**Manufacturing Date: 11MAY2016**

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	16 µg/mL 16 µg/mL 8 µg/mL <sup>7</sup> 5 µg/mL <sup>7</sup> 4 µg/mL 40 µg/mL 8 µg/mL <sup>8,9</sup> 16 µg/mL ≤ 0.5 µg/mL <sup>7</sup> 16 µg/mL <sup>7,9,10</sup> 16 µg/mL 32 µg/mL <sup>7,9,11</sup>
<b>Genotypic Analysis</b> Sequencing of Heat Shock Protein 65 gene (~ 1620 base pairs)  Digital DNA-DNA hybridization (dDDH) <sup>13</sup>	≥ 99% sequence identity to <i>M. tuberculosis</i> , strain XTB13-108 (GenBank: JLL001000001.1) ≥ 70% for species identification	100% sequence identity to <i>M. tuberculosis</i> , strain XTB13-108 (GenBank: JLL001000001.1) <sup>12</sup> <i>M. tuberculosis</i> (98.4%) <sup>14,15</sup> <i>M. microti</i> (98.3%) <sup>15</sup> <i>M. pinnipedii</i> (97.7%) <sup>15</sup> <i>M. africanum</i> (98.3%) <sup>15</sup> <i>M. caprae</i> (98.0%) <sup>15</sup> <i>M. canettii</i> (92.3%) <sup>15</sup> <i>M. bovis</i> (97.5%) <sup>15</sup> <i>M. mungii</i> (98.4%) <sup>15</sup> <i>M. orygis</i> (98.1%) <sup>15</sup>

TEST	SPECIFICATIONS	RESULTS
<b>Purity (post-freeze)</b> Middlebrook 7H10 agar with OADC enrichment <sup>16</sup>  Tryptic Soy agar <sup>17</sup>	Growth consistent with expected colony morphology Report results	Growth consistent with expected colony morphology No growth
<b>Viability (post-freeze)<sup>3</sup></b>	Growth	Growth

<sup>1</sup>NR-49365 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 62 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 moxifloxacin (4 µg/mL and 8 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 rifabutin (8 µg/mL and 16 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.

<sup>11</sup>Two MICs were observed for streptomycin (16 µg/mL and 32 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.

<sup>12</sup>Also consistent with *M. africanum*, *M. bovis*, *M. canettii*, *M. caprae* and *M. microti*

<sup>13</sup>Relatedness between bacterial strains has traditionally been determined using DDH. For additional information, refer to Auch, A. F., et al. "Digital DNA-DNA Hybridization for Microbial Species Delineation by Means of Genome-to-Genome Sequence Comparison." *Stand. Genomic Sci.* 2 (2010): 117-134. PubMed: 21304684.

<sup>14</sup>The whole genome of *M. tuberculosis*, strain XTB13-108 (Contig Total Length ~ 4.4 megabase pairs) was sequenced using the Illumina® MiSeq® system and was assembled and analyzed with CLC Genomics Workbench Version 7.0.2.

<sup>15</sup>Species within the *Mycobacterium tuberculosis* complex cannot be differentiated by DNA-DNA hybridization due to 90-100% DNA relatedness between the individual species (Imaeda, T. "Deoxyribonucleic Acid Relatedness Among Selected Strains of *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium bovis* BCG, *Mycobacterium microti*, and *Mycobacterium africanum*." *Int. J. Syst. Bacteriol.* 35 (1985): 147-150.).

<sup>16</sup>Purity of this lot was assessed for 93 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub>.

<sup>17</sup>Purity of this lot was assessed for 33 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub>.

Figure 1: Colony Morphology



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