

## Certificate of Analysis for NR-30780

## Mycobacterium tuberculosis, Strain 97-2476

## Catalog No. NR-30780

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

**Product Description:** *Mycobacterium tuberculosis (M. tuberculosis)*, strain 97-2476 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>: 64496458 Manufacturing Date: 17FEB2017

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis <sup>2</sup>		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology <sup>3</sup>	Report results	Irregular, slight peaked, undulate, rough and cream (Figure 1)
Growth rate	≥ 7 days	21 days
Growth at 26°C	Negative	Variable <sup>4</sup>
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>5</sup>	Positive	Positive
Nitrate reduction	Positive	Positive
Pyrazinamidase	Positive	Positive
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene (~ 440 base pairs)	≥ 99% sequence identity to <i>M. tuberculosis</i> type strain (GenBank: AL123456)	100% sequence identity to  M. tuberculosis type strain (GenBank: AL123456) <sup>6</sup>
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment <sup>7</sup>	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Tryptic Soy agar <sup>8</sup>	Report results	Growth consistent with expected colony morphology
Viability (post-freeze) <sup>3</sup>	Growth	Growth

<sup>&</sup>lt;sup>1</sup>NR-30780 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 66 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub> to produce this lot

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<sup>&</sup>lt;sup>2</sup>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://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacteria">https://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://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacterium">https://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacterium</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://www.intechopen.com/books/biochemical-testing/bio

<sup>321</sup> days at 37°C in an aerobic atmosphere with 5% CO₂ on Middlebrook 7H10 agar with OADC enrichment

<sup>&</sup>lt;sup>4</sup>Both growth and no growth were observed for this strain at 26°C in an aerobic atmosphere with 5% CO<sub>2</sub> in Middlebrook 7H9 broth with ADC enrichment. Specifications for this test were obtained from Bergey's Manual® of Systematic Bacteriology, 2<sup>nd</sup> ed., Volume 5, Part A, which indicates that no growth at 26°C (negative result) is represented by >90% of strains tested, and growth at 26°C (positive result) is represented by <10% of strains tested.

<sup>&</sup>lt;sup>5</sup>All mycobacteria produce niacin but only *M. tuberculosis* accumulates it, resulting in a positive test for *M. tuberculosis*.

<sup>&</sup>lt;sup>6</sup>Also consistent with M. africanum, M. bovis, M. canettii, M. caprae and M. microti

<sup>&</sup>lt;sup>7</sup>Purity of this lot was assessed for 33 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub>.

<sup>&</sup>lt;sup>8</sup>Purity of this lot was assessed for 21 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub>.



## **Certificate of Analysis for NR-30780**

Figure 1: Colony Morphology



/Heather Couch/

Heather Couch 04 SEP 2018

Program Manager or designee, ATCC Federal Solutions

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