

Certificate of Analysis for NR-30656

Mycobacterium tuberculosis, Strain 96-2397

Catalog No. NR-30656

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

Product Description: *Mycobacterium tuberculosis* (*M. tuberculosis*), strain 96-2397 was isolated between 1995 and 2000 from human sputum from an HIV-negative patient infected with pulmonary tuberculosis in North America. Strain 96-2397 was deposited as a multi-drug sensitive (MDS) strain of tuberculosis with sensitivity to rifampicin and isoniazid.

Lot¹: 63103796 Manufacturing Date: 12FEB2015

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ²		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ³	Report results	Irregular, low convex, undulate, rough and cream (Figure 1)
Growth rate	≥ 7 days	18 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 production⁴	Positive	Positive
Nitrate reduction	Positive	Positive
Pyrazinamidase	Positive	Positive
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene	Consistent with M. tuberculosis	Consistent with <i>M. tuberculosis</i> ⁵
(420 base pairs)		
Purity (post-freeze) ⁶	Consistent with expected colony morphology	Consistent with expected colony morphology
Viability (post-freeze) ³	Growth	Growth

NR-30656 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment and grown for 32 days at 37°C in an aerobic atmosphere with 5% CO₂. Broth inoculum was added to Middlebrook 7H10 agar with OADC enrichment kolles which were grown for 20 days at 37°C in an aerobic atmosphere with 5% CO₂ to produce this lot.

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²Information 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." <u>Int. J. Syst. Bacteriol.</u> 42 (1992): 315-323. PubMed: 1581193.

³18 days at 37°C in an aerobic atmosphere with 5% CO₂ on Middlebrook 7H10 agar with OADC enrichment

⁴All mycobacteria produce niacin but only *M. tuberculosis* accumulates it, resulting in a positive test for *M. tuberculosis*.

⁵Also consistent with *M. africanum*, *M. bovis*, *M. canettii* and *M. microti*

⁶Purity of this lot was assessed for 18 days at 37°C in an aerobic atmosphere with 5% CO₂ on Middlebrook 7H10 agar with OADC enrichment and on Tryptic Soy agar plates.



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Figure 1: Colony Morphology



Date: 21 JAN 2016

Signature:

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