

Certificate of Analysis for NR-30617

Mycobacterium tuberculosis, Strain 96-2068

Catalog No. NR-30617

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

Product Description: *Mycobacterium tuberculosis* (*M. tuberculosis*), strain 96-2068 was isolated between 1995 and 2000 from human sputum from an HIV-negative patient infected with pulmonary tuberculosis in North America. Strain 96-2068 was deposited as a multi-drug sensitive (MDS) strain of tuberculosis with sensitivity to rifampicin and isoniazid.

Lot¹: 63101907

Manufacturing Date: 22JAN2015

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis² Cellular morphology Colony morphology ³ 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 ⁴ 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) 19 days Negative Positive Positive (red colonies) Negative (no pigment) Negative (no pigment) Positive (no pigment) Positive Positive Positive
Genotypic Analysis Sequencing of Heat Shock Protein 65 gene (~ 440 base pairs)	Consistent with <i>M. tuberculosis</i>	Consistent with <i>M. tuberculosis</i> ⁵
Purity (post-freeze)⁶	Consistent with expected colony morphology	Consistent with expected colony morphology
Viability (post-freeze)³	Growth	Growth

¹NR-30617 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment and grown for 30 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 34 days at 37°C in an aerobic atmosphere with 5% CO₂ to produce this lot.

²Information 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.

³19 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 21 days at 37°C in an aerobic atmosphere with 5% CO₂ on Middlebrook 7H10 agar with OADC enrichment and Tryptic Soy agar plates.

Figure 1: Colony Morphology

**Date:** 26 JAN 2016**Signature:**

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