

Certificate of Analysis for NR-30627

Mycobacterium tuberculosis, Strain 96-2216

Catalog No. NR-30627

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

Product Description: *Mycobacterium tuberculosis* (*M. tuberculosis*), strain 96-2216 was isolated between 1995 and 2000 from human sputum from an HIV-negative patient infected with pulmonary tuberculosis in North America. Strain 96-2216 was deposited as a multi-drug sensitive (MDS) strain of tuberculosis with sensitivity to rifampicin and isoniazid.

Lot¹: 63101929 Manufacturing Date: 22JAN2015

| TEST | SPECIFICATIONS | RESULTS |
|---|--|--|
| Phenotypic Analysis ² | | |
| Cellular morphology | Gram-positive rods | Gram-positive rods |
| Colony morphology ³ | Report results | Irregular, slight peaked, undulate, rough and cream (Figure 1) |
| Growth rate | ≥ 7 days | 20 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 production ⁴ | Positive | Positive |
| Nitrate reduction | Positive | Positive |
| Pyrazinamidase | Positive | Positive |
| Genotypic Analysis | | |
| Sequencing of Heat Shock Protein 65 gene (~ 430 base pairs) | Consistent with M. tuberculosis | 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-30627 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.

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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." Int. J. Syst. Bacteriol. 42 (1992): 315-323. PubMed: 1581193.

³20 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.



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Figure 1: Colony Morphology



Date: 01 FEB 2016

Signature:

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