

Certificate of Analysis for NR-30701

Mycobacterium tuberculosis, Strain 96-2992

Catalog No. NR-30701

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

Product Description: Mycobacterium tuberculosis (M. tuberculosis), strain 96-2992 was isolated between 1995 and 2000 from human sputum from an HIV-negative patient infected with pulmonary tuberculosis in North America. Strain 96-2992 was deposited as a multi-drug sensitive (MDS) strain of tuberculosis with sensitivity to rifampicin and isoniazid.

Lot1: 63385547 Manufacturing Date: 16JUN2015

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ²		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ³	Report results	Irregular, slight peaked, undulate, opaque, rough and cream (Figure 1)
Growth rate	≥ 7 days	21 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 (~ 420 base pairs)	≥ 99% sequence identity to <i>M. tuberculosis</i> type strain (GenBank: AL123456)	100% sequence identity to M. tuberculosis type strain (GenBank: AL123456) ⁵
Purity (post-freeze) ⁶	Consistent with expected colony morphology	Consistent with expected colony morphology
Viability (post-freeze) ³	Growth	Growth

¹NR-30701 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 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." Biochemical Testing. (2012) Jose C. Jimenez-Lopez (Ed.), InTech, <a href="http://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-decomposition-and-identification-and-identification-of-decomposition-and-identification-and-identifi 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. ³21 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. microti* and *M. canettii*

⁶Purity of this lot was assessed for 44 days at 37°C in an aerobic atmosphere with 5% CO₂ on Middlebrook 7H10 agar with OADC enrichment and for 30 days at 37°C in an aerobic atmosphere with 5% CO₂ on Tryptic Soy agar plates.



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Figure 1: Colony Morphology



Date: 04 MAY 2016

Signature:

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