

Certificate of Analysis for NR-30688

Mycobacterium tuberculosis, Strain 96-2729

Catalog No. NR-30688

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

Product Description: *Mycobacterium tuberculosis* (*M. tuberculosis*), strain 96-2729 was isolated between 1995 and 2000 from human sputum from an HIV-negative patient infected with pulmonary tuberculosis in North America. Strain 96-2729 was deposited as a multi-drug sensitive (MDS) strain of tuberculosis with sensitivity to rifampicin and isoniazid.

Lot¹: 63385534 Manufacturing Date: 26JUN2015

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ²		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ³	Report results	Irregular, raised, undulate, rough and cream (Figure 1)
Growth rate	≥ 7 days	31 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 (~ 400 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-30688 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 25 days at 37°C in an aerobic atmosphere with 5% CO₂ to produce this lot.

BEI Resources www.beiresources.org E-mail: contact@beiresources.org

Fax: 703-365-2898 Tel: 800-359-7370

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.

³³¹ 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 48 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: 26 JUL 2016

Signature: (

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