Certificate of Analysis for NR-30681

Mycobacterium tuberculosis, Strain 96-2626

Catalog No. NR-30681

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

Product Description:

Mycobacterium tuberculosis (M. tuberculosis), strain 96-2626 was isolated between 1995 and 2000 from human sputum from an HIV-negative patient infected with pulmonary tuberculosis in North America. Strain 96-2626 deposited as a drug-sensitive strain of tuberculosis with sensitivity to rifampicin and isoniazid. NR-30681 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 17 days at 37°C in an aerobic atmosphere with 5% CO_2 to produce this lot.

Lot: 63385527 **Manufacturing Date: 18JUN2015**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[1]		
Cellular morphology 21 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment	Gram-positive rods	Gram-positive rods
Colony morphology 21 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment	Report results	Irregular, low convex, undulate, rough and cream
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[2]	Positive	Positive
Nitrate reduction	Positive	Positive
Pyrazinamidase	Positive	Positive
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene (~ 430 base pairs)	≥ 99% sequence identity to *M. tuberculosis* type strain (GenBank: AL123456.1)	100% sequence identity to *M. tuberculosis* type strain (GenBank: AL123456.1)[3]
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment 36 days at 37°C in an aerobic atmosphere with 5% CO_2	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Tryptic Soy agar 21 days at 37°C in an aerobic atmosphere with 5% CO_2	Report results	Growth consistent with expected colony morphology
Viability (post-freeze) 21 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment	Growth	Growth

[1]Information 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.
[2]All mycobacteria produce niacin but only *M. tuberculosis* accumulates it, resulting in a positive test for *M. tuberculosis*.
[3]Also consistent with other members of the *M. tuberculosis* complex.

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/Heather Couch/
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Program Manager or designee, ATCC Federal Solutions

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