b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-30731

Mycobacterium tuberculosis, Strain 96-3270

Catalog No. NR-30731

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

Product Description: *Mycobacterium tuberculosis* (*M. tuberculosis*), strain 96-3270 was isolated between 1995 and 2000 from human sputum from an HIV-negative patient infected with pulmonary tuberculosis in North America. Strain 96-3270 was deposited as a multi-drug sensitive (MDS) strain of tuberculosis with sensitivity to rifampicin and isoniazid.

Lot[1]: 64121896 **Manufacturing Date: 20MAY2016**

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	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 (~440 base pairs)	≥ 99% sequence identity to *M. tuberculosis* type strain (GenBank: AL123456)	100% sequence identity to *M. tuberculosis* type strain (GenBank: AL123456)[5]
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment[6]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Tryptic Soy agar[7]	No Growth	No Growth
Viability (post-freeze)[3]	Growth	Growth

[1]NR-30731 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 65 days at 37°C in an aerobic atmosphere with 5% CO_2 to produce this lot.

[2]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.

[3]21 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment

[4]All mycobacteria produce niacin but only *M. tuberculosis* accumulates it, resulting in a positive test for *M. tuberculosis*.

[5]Also consistent with *M. africanum*, *M. bovis*, *M. canettii* and *M. microti*

[6]Purity of this lot was assessed for 53 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment.

[7]Purity of this lot was assessed for 21 days at 37°C in an aerobic atmosphere with 5% CO_2 on Tryptic Soy agar plates.

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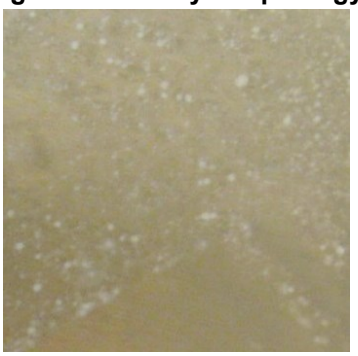
Figure 1: Colony Morphology



Date: 04 JAN 2017 **Signature:**

BEI Resources Authentication

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