b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-30653

Mycobacterium tuberculosis, Strain 96-2365

Catalog No. NR-30653

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

Product Description:

Mycobacterium tuberculosis (M. tuberculosis), strain 96-2365 was isolated between 1995 and 2000 from human sputum from an HIV-negative patient with drug-susceptible tuberculosis in North America. Strain 96-2365 deposited as a drug-sensitive strain of tuberculosis with sensitivity to rifampicin and isoniazid. NR-30653 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment and grown for 35 days at 37°C in an aerobic atmosphere with 5% CO_2. 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% CO_2 to produce this lot.

Lot: 63103787 **Manufacturing Date: 12FEB2015**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[1]		
Cellular morphology 18 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 18 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	18 days
Growth at 26°C	Negative	Negative
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 (~ 420 base pairs)	≥ 99% sequence identity to *M. tuberculosis* type strain (GenBank: AL123456)	100% sequence identity to *M. tuberculosis* type strain (GenBank: AL123456)[3]
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment 18 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 18 days at 37°C in an aerobic atmosphere with 5% CO_2	Report results	Growth consistent with expected colony morphology
Viability (post-freeze) 18 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, 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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