b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-19004

Mycobacterium tuberculosis, Strain HN3226

Catalog No. NR-19004

Product Description: *Mycobacterium tuberculosis (M. tuberculosis)*, strain HN3226 was isolated in 1991 in Harris, Texas, USA. Strain HN3226 was deposited as a rifabutin-resistant strain.

Lot[1]: 63344542 **Manufacturing Date: 20MAR2015**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[2]		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology[3]	Report results	Irregular, slight peaked, undulate, rough, opaque and cream (Figure 1)
Growth rate	≥ 7 days	30 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
Antibiotic Susceptibility Profile		
Sensititre™ System[5,6]		
Amikacin	Report results	≤ 0.12 µg/mL
Cycloserine	Report results	8 µg/mL
Ethambutol	Report results	1 µg/mL[7]
Ethionamide	Report results	1.2 µg/mL[7]
Isoniazid	Report results	≤ 0.03 µg/mL
Kanamycin	Report results	≤ 0.6 µg/mL
Moxifloxacin	Report results	0.5 µg/mL
Ofloxacin	Report results	1.0 µg/mL
Para-aminosalicylic acid	Report results	≤ 0.5 µg/mL[7]
Rifabutin	Resistant	≤ 0.12 µg/mL[7]
Rifampin	Report results	≤ 0.12 µg/mL
Streptomycin	Report results	≤ 0.25 µg/mL[7]
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)[8]
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment[9]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Tryptic Soy agar[9]	Report results	No growth
Viability (post-freeze)[3]	Growth	Growth

[1]NR-19004 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 23 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.

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[3]30 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]Sensititre™ System *Mycobacterium tuberculosis* MIC Plate, Thermo Scientific™, catalog number MYCOTB
[6]Minimum Inhibitory Concentration (MIC); No Clinical & Laboratory Standards Institute (CLSI) interpretations of the Sensititre™ System data for *M. tuberculosis* are currently available
[7]For streptomycin, ethionamide, para-aminosalicylic acid, rifabutin and ethambutol, the endpoint for these drugs is determined by the well with approximately 80% inhibition of growth compared to the positive control well with no drug.
[8]Also consistent with *M. africanum*, *M. bovis*, *M. canettii, M. caprae* and *M. microti*
[9]Purity of this lot was assessed for 40 days at 37°C in an aerobic atmosphere with 5% CO_2.

Figure 1: Colony Morphology

Heather Couch

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15 FEB 2018

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