b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-30604

Mycobacterium tuberculosis, Strain 95-2457

Catalog No. NR-30604

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

Product Description: *Mycobacterium tuberculosis* (*M. tuberculosis*), strain 95-2457 was isolated between 1995 and 2000 from human sputum from an HIV-negative patient infected with pulmonary tuberculosis in North America. Strain 95-2457 was deposited as a multi-drug sensitive (MDS) strain of tuberculosis with sensitivity to rifampicin and isoniazid.

Lot[1]: 61255110 **Manufacturing Date: 20NOV2012**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[2]		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology[3]	Report results	Circular, peaked, rough and white (Figure 1)
Growth on Brain Heart Infusion agar	Report results	Growth
Growth on MacConkey agar (without crystal violet)	No growth	No growth
Growth rate	≥ 7 days	21 days
Growth at 26°C	Negative	Negative
Growth at 37°C	Positive	Positive
Growth at 45°C	Negative	Negative
Growth at 55°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[4]	Positive	Positive
Nitrate reduction	Positive	Positive
Pyrazinamidase	Positive	Positive
Urease[5]	Report results	Positive
Aryl sulfate (3 days)	Negative	Negative
Aryl sulfate (14 days)	Positive	Negative[6]
Catalase	Positive	Positive
Iron uptake	Negative	Negative
Tween 80 hydrolysis	Report results	Negative
Growth in the presence of 5% sodium chloride	Negative	Negative
Growth in the presence of thiophene-2-carboxylic acid hydrazide (TCH)	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)[7]
Purity (post-freeze)[8,9]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze)[10]	Growth	Growth

[1]NR-30604 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment and grown for 16 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.

[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-

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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]39 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]>85% of *M. tuberculosis* strains are positive for urease activity.
[6]Most slow-growing *M. tuberculosis* test positive for aryl sulfate after 14 days, but very slow growers may still show a negative result.
[7]Also consistent with *M. africanum*, *M. bovis* and *M. microti*
[8]Purity of this lot was assessed for 28 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar.
[9]Middlebrook 7H10 agar with OADC enrichment contains malachite green, which may inhibit growth of contaminating microorganisms.
[10]21 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment

Figure 1: Colony Morphology

Date: 27 SEP 2016

Signature:

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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