

## Certificate of Analysis for NR-44274

## Mycobacterium abscessus subsp. abscessus, Strain 4530

## Catalog No. NR-44274

**Product Description:** *Mycobacterium abscessus (M. abscessus)* subsp. *abscessus*, strain 4530 was isolated between 2009 and 2013 from human sputum in Texas, USA.

Lot<sup>1</sup>: 64446906 Manufacturing Date: 15AUG2016

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis <sup>2,3</sup>		
Cellular morphology	Report results	Rods
Colony morphology <sup>4</sup>	Report results	Irregular, convex, undulate, rough and white
Growth on MacConkey agar (without crystal violet) Growth rate Growth at 45°C Growth at 55°C Acid-fast stain Pigmentation in the dark (Scotochromogen) Photoinduction for 1 hour (Photochromogen) Nonchromogen (no pigment) Biochemical tests Nitrate reduction Urease Catalase Catalase Catalase (semiquantitative) Catalase (68°C) Iron uptake Tween 80 hydrolysis Growth in the presence of 5% sodium chloride	Positive ≤ 7 days Negative Report results Positive (red colonies) Negative (no pigment) Negative (no pigment) Positive (no pigment)  Negative Positive Report results Report results Report results Negative Negative Negative Positive	Positive 4 days Positive <sup>5</sup> Negative Positive (red colonies) Negative (no pigment) Negative (no pigment) Positive (no pigment)  Negative Positive Positive Positive Positive Negative Negative Negative Negative Positive Positive Positive Positive Positive Positive Positive Positive
Growth in the presence of thiophene-2-carboxylic acid hydrazide (TCH)	Report results	Positive
Genotypic Analysis <sup>6</sup> Sequencing of 16S ribosomal RNA gene (~ 1070 base pairs)  Digital DNA-DNA hybridization (dDDH)	≥ 99% sequence identity to <i>M. abscessus</i> subsp. <i>abscessus</i> type strain (GenBank: X82235.1) ≥ 70% for species identification	100% sequence identity to M. abscessus subsp. abscessus type strain (GenBank: X82235.1) M. abscessus subsp. abscessus (99.8%) <sup>7,8</sup> M. abscessus subsp. bolleti (77.3%) M. abscessus subsp. massiliense (75.2%)
Purity (post-freeze) Middlebrook 7H10 agar with OADC enrichment <sup>9</sup> Tryptic Soy agar <sup>9</sup>	Growth consistent with expected colony morphology Report results	Growth consistent with expected colony morphology Growth consistent with expected colony morphology
Viability (post-freeze) <sup>4</sup>	Growth	Growth

<sup>&</sup>lt;sup>1</sup>NR-44274 was produced by inoculation of the deposited material in Middlebrook 7H9 broth with ADC enrichment for 14 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub>. Broth inoculum was added to Middlebrook 7H10 agar with OADC enrichment kolles, which were grown for 5 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub> to produce this lot.

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<sup>&</sup>lt;sup>2</sup>Information on Mycobacterium testing is available from Ribón, W. "Biochemical Isolation and Identification of Mycobacteria." <u>Biochemical Testing</u>. (2012) Jose C. Jimenez-Lopez (Ed.), InTech, <a href="http://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacteria">http://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacteria</a> and Lévy-Frébault, V. V. and F. Portaels. "Proposed Minimal Standards for the Genus *Mycobacterium* and for Description of New Slowly Growing *Mycobacterium* Species." <a href="https://example.com/intention-new-april 1992">https://example.com/intention-new-april 1992</a> and Lévy-Frébault, V. V. and F. Portaels. "Proposed Minimal Standards for the Genus *Mycobacterium* and for Description of New Slowly Growing *Mycobacterium* Species." <a href="https://example.com/intention-new-april 1992">https://example.com/intention-new-april 1992</a> and Lévy-Frébault, V. V. and F. Portaels. "Proposed Minimal Standards for the Genus *Mycobacterium* and for Description of New Slowly Growing *Mycobacterium* Species." <a href="https://example.com/intention-new-april 1992">https://example.com/intention-new-april 1992</a> and 1992 an



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<sup>3</sup>Phenotypic characterization of *M. abscessus* subsp. *abscessus* was performed following: Kusunoki, S. and T. Ezaki. "Proposal of *Mycobacterium peregrinum* sp. nov., nom. rev., and Elevation of *Mycobacterium chelonae* subsp. *abscessus* (Kubica et al.) to Species Status: *Mycobacterium abscessus* comb. nov." <u>Int. J. Syst. Bacteriol.</u> 42 (1992): 240-245. PubMed: 1581184.

<sup>4</sup>4 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub> on Middlebrook 7H10 agar with OADC enrichment.

<sup>5</sup>Specification for this test was obtained from Magee, J. G. and A. C. Ward. "Family III. *Mycobacteriaceae* Chester 1897, 63<sup>AL</sup>." <u>Bergey's® Manual of Systematic Bacteriology, Second Edition, Volume Five.</u> (2012) Goodfellow, M., et al. (Ed.), Springer, which indicates that most strains of *M. abscessus* are negative for this test; however up to 10% of strains may be positive.

<sup>6</sup>Relatedness between bacterial strains has traditionally been determined using dDDH. For additional information refer to Auch, A.F., et al. "Digital DNA-DNA Hybridization for Microbial Species Delineation by Means of Genome-to-Genome Sequence Comparison." <u>Stand Genomic Sci.</u> 2 (2010): 117-134, PubMed: 21304684.

<sup>7</sup>The whole genome of *M. abscessus subsp. abscessus*, strain 4530 (~ 5.2 megabase pairs) was sequenced using the Illumina® MiSeq® system and was assembled and analyzed with CLC Genomics Workbench Version 7.0.2.

<sup>8</sup>Originally deposited as *M. xenopi* and updated to *M. abscessus* subsp. *abscessus* following dDDH analysis.

<sup>9</sup>Purity of this lot was assessed for 14 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub>.

**Date:** 31 MAY 2017

Signature:

**BEI Resources Authentication** 

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