

***Mycobacterium heraklionense*, Strain GN-1T**

Catalog No. NR-49082

Product Description: *Mycobacterium heraklionense* (*M. heraklionense*), strain GN-1T is a clinical isolate collected in 2002 from sputum of a 74-year old male in Greece.

Lot¹: 64362424

Manufacturing Date: 02AUG2016

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis^{2,3} Cellular morphology Colony morphology ⁴ 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 Catalase Catalase (semiquantitative) Catalase (68°C) Iron uptake Nitrate reduction Tween 80 hydrolysis Urease Growth in the presence of 5% sodium chloride Growth in the presence of thiophene-2-carboxylic acid hydrazide (TCH)	Rods Report results ≥ 7 days Negative Report results Positive (red colonies) Negative (no pigment) Negative (no pigment) Positive Report results Positive Positive Report results Positive Positive Negative Report results Positive	Rods Circular, low convex, entire, smooth and cream (Figure 1) 11 days Negative Negative Positive (red colonies) Negative (no pigment) Negative (no pigment) Positive Positive Positive Positive Negative Negative ⁵ Positive Negative Negative Positive
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~1430 base pairs) Digital DNA-DNA hybridization (dDDH) ⁶	≥ 99% sequence identity to <i>M. heraklionense</i> type strain (GenBank: GU084182.2) ≥ 70% for species identification	100% sequence identity to <i>M. heraklionense</i> type strain ⁷ (GenBank: GU084182.2) Not determined ^{8,9} (Table 1)
Purity (post-freeze) Middlebrook 7H10 agar with OADC enrichment ¹⁰ Tryptic Soy agar ¹⁰	Growth consistent with expected colony morphology Report results	Growth consistent with expected colony morphology Growth consistent with expected colony morphology
Viability (post-freeze)³	Growth	Growth

¹NR-49082 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment and grown for 14 days at 37°C in an aerobic atmosphere with 5% CO₂. Broth inoculum was added to Middlebrook 7H10 agar with OADC enrichment kolles, which were grown for 7 days at 37°C in an aerobic atmosphere with 5% CO₂ to produce this lot.

²Information 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.

³Phenotypic characterization of *M. heraklionense* was performed following Tortoli, E., et al. "Survey of 150 Strains Belonging to the *Mycobacterium terrae* Complex and Description of *Mycobacterium engbaekii* sp. nov., *Mycobacterium heraklionense* sp. nov. and *Mycobacterium heraklionense* sp. nov." *Int. J. Syst. Evol. Microbiol.* 63 (2013): 401-411. PubMed: 22447702.

⁴11 days at 37°C in an aerobic atmosphere with 5% CO₂ on Middlebrook 7H10 agar with OADC enrichment

⁵NR-49082 was deposited as *M. heraklionense* and reported to be positive for nitrate reduction. Testing performed in triplicate by BEI Resources indicates a negative result.

⁶Relatedness between bacterial strains has traditionally been determined using DDH. 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." *Stand. Genomic Sci.* 2 (2010):

117-134. PubMed: 21304684.

⁷*M. heraklionense*, strain GN-1T is also referred to as strain NCTC 13432^T (Tortoli, E., et al. "Survey of 150 Strains Belonging to the *Mycobacterium terrae* Complex and Description of *Mycobacterium engbaekii* sp. nov., *Mycobacterium heraklionense* sp. nov. and *Mycobacterium longobardum* sp. nov." *Int. J. Syst. Evol. Microbiol.* 63 (2013): 401-411. PubMed: 22447702.).

⁸The whole genome of *M. heraklionense*, strain GN-1T (Contig Total Length ~ 5.0 megabase pairs) was sequenced using the Illumina[®] MiSeq[®] system and was assembled and analyzed with CLC Genomics Workbench Version 7.0.2.

⁹The required whole genome sequence for the type strain of this species is not available. dDDH testing rules out all species listed in Table 1, however, this does not rule out species for which the type strains whole genome sequences are not available.

¹⁰Purity of this lot was assessed for 11 days at 37°C in an aerobic atmosphere with 5% CO₂.

Figure 1: Colony Morphology



Table 1: Digital DNA-DNA hybridization (dDDH)

Species	Strain	Accession #	GGD vs. NR-49082 (Deposited as: <i>M. heraklionense</i>)
<i>M. abscessus</i> subsp. <i>abscessus</i>	Hauduroy L948 ^T	NC_010397.1	22
<i>M. abscessus</i> subsp. <i>bolletii</i>	BD ^T	AHAS00000000.1	19.5
<i>M. abscessus</i> subsp. <i>massiliense</i>	CCUG 48898 ^T	NZ_AP014547.1	19.6
<i>M. aromaticivorans</i>	JS19b1 ^T	JALN00000000.2	20
<i>M. aurum</i>	ATCC 23366 ^T	CVQQ01000001.1	20.3
<i>M. austroafricanum</i>	E9789-SA12441 ^T	HG964450.1	19.2
<i>M. avium</i> subsp. <i>avium</i>	ATCC 25291 ^T	ACFI00000000.1	21.1
<i>M. avium</i> subsp. <i>paratuberculosis</i>	ATCC 19698 ^T	AGAR00000000.1	21.6
<i>M. avium</i> subsp. <i>silvaticum</i>	6409 ^T	AYOC00000000.1	21.5
<i>M. bohemicum</i>	CIP 105808 ^T	CSTD01000001.1	20.7
<i>M. canariense</i>	502329 ^T	BCSY00000000.1	20.2
<i>M. celatum</i>	ATCC 51131 ^T	BBUN00000000.1	21.3
<i>M. chelonae</i>	CM 6388 ^T	CP010946.1	19.5
<i>M. chlorophenicolum</i>	PCP-I ^T	JYNL00000000.1	20.8
<i>M. chubuense</i>	48013 ^T	NC_018027.1	20.1
<i>M. colombiense</i>	10B ^T	AFVW00000000.2	20.8
<i>M. conceptionense</i>	D16 ^T	CTEF00000000.1	21.3
<i>M. cosmeticum</i>	LTA-388 ^T	CCBB00000000.1	20.9
<i>M. crocinum</i>	czh-42 ^T	BBHD00000000.1	21.5
<i>M. farcinogenes</i>	IEMVT 75 ^T	CCAY00000000.1	20.2
<i>M. fluoranthenvivorans</i>	FA4 ^T	BBFT00000000.1	21.4
<i>M. fortuitum</i> subsp. <i>fortuitum</i>	ATCC 6841 ^T	CP014258.1	20
<i>M. fortuitum</i> subsp. <i>acetamidolyticum</i>	NCH E11620 ^T	BCSZ00000000.1	21
<i>M. gastri</i>	ATCC 15754 ^T	AZYN00000000.1	20.6
<i>M. genavense</i>	2289 ^T	JAGZ00000000.1	20.4
<i>M. haemophilum</i>	ATCC 29548 ^T	CP011883.2	20

Species	Strain	Accession #	GGD vs. NR-49082 (Deposited as: <i>M. heraklionense</i>)
<i>M. hassiacum</i>	3849 ^T	ARBU00000000.1	20.2
<i>M. hodleri</i>	EMI2 ^T	BBGO00000000.1	22.4
<i>M. intracellulare</i>	ATCC 13950 ^T	NC_016946.1	20.7
<i>M. kansasii</i>	ATCC 12478 ^T	NC_022663.1	20.3
<i>M. kyorinense</i>	KUM 060204 ^T	BBKA00000000.1	20.6
<i>M. mageritense</i>	938 ^T	CCBF00000000.1	20
<i>M. neoaurum</i>	ATCC 25795 ^T	JMDW00000000.1	19.9
<i>M. neworleansense</i>	W6705 ^T	CWKH00000000.1	20.1
<i>M. novocastrense</i>	73 ^T	BCTA00000000.1	20.9
<i>M. obuense</i>	47001 ^T	JYNU00000000.1	19.8
<i>M. pallens</i>	czh-8 ^T	BBHE00000000.1	21.5
<i>M. parascrofulaceum</i>	HSC-68 ^T	ADNV00000000.1	21.6
<i>M. pseudoshottsii</i>	L15 ^T	BCND00000000.1	20.2
<i>M. pyrenivorans</i>	17A3 ^T	BBHB00000000.1	23.5
<i>M. rufum</i>	JS14 ^T	JROA00000000.1	20.7
<i>M. rutilum</i>	czh-117 ^T	BBHF00000000.1	23
<i>M. septicum</i>	W4964 ^T	CBMO00000000.1	20.2
<i>M. setense</i>	ABO-M06 ^T	JTJW00000000.1	20
<i>M. simiae</i>	ATCC 25275 ^T	CBMJ00000000.2	20.2
<i>M. smegmatis</i>	ATCC 19420 ^T	LN831039.1	20.1
<i>M. thermoresistibile</i>	ATCC 19527 ^T	BCTB00000000.1	20.3
<i>M. triplex</i>	90-1019 ^T	CCAU00000000.1	20.6
<i>M. tuberculosis</i>	H37Rv ^T	NC_000962.3	20.1
<i>M. vaccae</i>	ATCC 15483 ^T	B CRS00000000.1	20.4
<i>M. vanbaalenii</i>	PYR-1 ^T	NC_008726.1	20.2
<i>M. vulneris</i>	NLA000700772 ^T	CCBG00000000.1	20.4
<i>M. yongonense</i>	05-1390 ^T	NC_021715.1	20.7
<i>Nocardia asteroides</i>	NBRC 15531 ^T	BAFO00000000.2	19.5

Date: 13 JUN 2017

Signature:



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