

***Mycobacterium fragae*, Strain HF8705T**
Catalog No. NR-49084
Product Description: *Mycobacterium fragae* (*M. fragae*), strain HF8705T was isolated in 2010 from the sputum of a patient with a lung infection in Ceará, Brazil.

Lot¹: 64362419
Manufacturing Date: 04AUG2016

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) Growth on MacConkey agar without crystal violet	Rods Report results ≥ 7 days Negative Report results Positive (red colonies) Negative (no pigment) Negative (no pigment) Positive Report results Negative Positive Report results Negative Negative Negative Negative Report results Positive Negative	Rods Circular, low convex, entire, rough and white ⁵ (Figure 1) 17 days Positive ⁶ Negative Positive (red colonies) Negative (no pigment) Negative (no pigment) Positive Positive Negative Positive Negative Negative Negative Negative Negative Negative Negative ⁷ Negative
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~1450 base pairs) Digital DNA-DNA hybridization (dDDH) ⁸	≥ 99% sequence identity to <i>M. fragae</i> type strain (GenBank: JQ898451.1) ≥ 70% for species identification	100% sequence identity to <i>M. fragae</i> type strain (GenBank: JQ898451.1) Not determined ^{9,10} (Table 1)
Purity (post-freeze) Middlebrook 7H10 agar with OADC enrichment ¹¹ Tryptic Soy agar ¹¹	Growth consistent with expected colony morphology Report results	Growth consistent with expected colony morphology Growth consistent with expected colony morphology
Viability (post-freeze)⁴	Growth	Growth

¹NR-49084 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment and grown for 15 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 9 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. fragae* was performed following Ramos, J. P., et al. "*Mycobacterium fragae* sp. nov., a Non-Chromogenic Species Isolated from Human Respiratory Specimens." *Int. J. Syst. Evol. Microbiol.* 63 (2013): 2583-2587. PubMed: 23264503.

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

⁵NR-49084 was deposited as *M. fragae* and reported to produce colonies with a smooth morphology. Testing performed in duplicate by BEI Resources indicates colonies produced a rough morphology.

⁶NR-49084 was deposited as *M. fragae* and reported to be negative for growth at 45°C. Testing performed in triplicate by BEI Resources indicates a positive result.

⁷NR-49084 was deposited as *M. fragae* and reported to be positive for growth in the presence of TCH. Testing performed in duplicate by BEI Resources indicates a negative result.

⁸Relatedness 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.

⁹The whole genome of *M. fragae*, strain HF8705T (Contig Total Length ~4.7 megabase pairs) was sequenced using the Illumina® MiSeq® system and was assembled and analyzed with CLC Genomics Workbench Version 7.0.2.

¹⁰The 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.

¹¹Purity of this lot was assessed for 17 days at 37°C in an aerobic atmosphere with 5% CO₂.

Figure 1: Colony Morphology

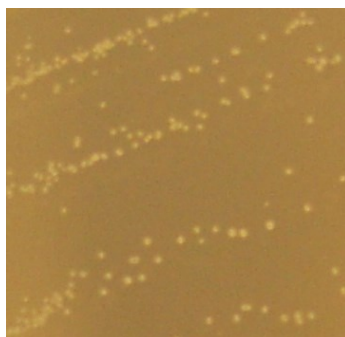


Table 1: Digital DNA-DNA hybridization (dDDH)

Species	Strain	Accession #	GGD vs. NR-49084 (Deposited as: <i>M. fragae</i>)
<i>M. abscessus</i> subsp. <i>abscessus</i>	Hauduroy L948 ^T	NC_010397.1	19.4
<i>M. abscessus</i> subsp. <i>bolletii</i>	BD ^T	AHAS00000000.1	19.4
<i>M. abscessus</i> subsp. <i>massiliense</i>	CCUG 48898 ^T	NZ_AP014547.1	19.2
<i>M. aromaticivorans</i>	JS19b1 ^T	JALN00000000.2	19.6
<i>M. aurum</i>	ATCC 23366 ^T	CVQQ01000001.1	19.4
<i>M. austroafricanum</i>	E9789-SA12441 ^T	HG964450.1	19.4
<i>M. avium</i> subsp. <i>avium</i>	ATCC 25291 ^T	ACFI00000000.1	22.1
<i>M. avium</i> subsp. <i>paratuberculosis</i>	ATCC 19698 ^T	AGAR00000000.1	22.7
<i>M. avium</i> subsp. <i>silvaticum</i>	6409 ^T	AYOC00000000.1	22.5
<i>M. bohemicum</i>	CIP 105808 ^T	CSTD01000001.1	22.3
<i>M. canariense</i>	502329 ^T	BCSY00000000.1	19.6
<i>M. celatum</i>	ATCC 51131 ^T	BBUN00000000.1	25.5
<i>M. chelonae</i>	CM 6388 ^T	CP010946.1	19.4
<i>M. chlorophenicolum</i>	PCP-I ^T	JYNL00000000.1	19.5
<i>M. chubuense</i>	48013 ^T	NC_018027.1	19.5
<i>M. colombiense</i>	10B ^T	AFVW00000000.2	21.6
<i>M. conceptionense</i>	D16 ^T	CTEF00000000.1	19.5
<i>M. cosmeticum</i>	LTA-388 ^T	CCBB00000000.1	19.7
<i>M. crocinum</i>	czh-42 ^T	BBHD00000000.1	21.2
<i>M. farcinogenes</i>	IEMVT 75 ^T	CCAY00000000.1	19.3
<i>M. fluoranthivorans</i>	FA4 ^T	BBFT00000000.1	20.8
<i>M. fortuitum</i> subsp. <i>fortuitum</i>	ATCC 6841 ^T	CP014258.1	19.4
<i>M. fortuitum</i> subsp. <i>acetamidolyticum</i>	NCH E11620 ^T	BCSZ00000000.1	19.6
<i>M. gastri</i>	ATCC 15754 ^T	AZYN00000000.1	22

Certificate of Analysis for NR-49084

Species	Strain	Accession #	GGD vs. NR-49084 (Deposited as: <i>M. fragae</i>)
<i>M. genavense</i>	2289 ^T	JAGZ00000000.1	21.6
<i>M. haemophilum</i>	ATCC 29548 ^T	CP011883.2	21.3
<i>M. hassiacum</i>	3849 ^T	ARBU00000000.1	20
<i>M. hodleri</i>	EMI2 ^T	BBGO00000000.1	22.1
<i>M. intracellulare</i>	ATCC 13950 ^T	NC_016946.1	21.6
<i>M. kansasii</i>	ATCC 12478 ^T	NC_022663.1	21.6
<i>M. kyorinense</i>	KUM 060204 ^T	BBKA00000000.1	25
<i>M. mageritense</i>	938 ^T	CCBF00000000.1	19.7
<i>M. neoaurum</i>	ATCC 25795 ^T	JMDW00000000.1	19.1
<i>M. neworleansense</i>	W6705 ^T	CWKH00000000.1	19.5
<i>M. novocastrense</i>	73 ^T	BCTA00000000.1	19.8
<i>M. obuense</i>	47001 ^T	JYNU00000000.1	19.3
<i>M. pallens</i>	czh-8 ^T	BBHE00000000.1	21.5
<i>M. parascrofulaceum</i>	HSC-68 ^T	ADNV00000000.1	22.1
<i>M. pseudoshottsii</i>	L15 ^T	BCND00000000.1	20.9
<i>M. pyrenivorans</i>	17A3 ^T	BBHB00000000.1	21.6
<i>M. rufum</i>	JS14 ^T	JROA00000000.1	19.5
<i>M. rutilum</i>	czh-117 ^T	BBHF00000000.1	23.3
<i>M. septicum</i>	W4964 ^T	CBMO00000000.1	19.6
<i>M. setense</i>	ABO-M06 ^T	JTJW00000000.1	19.4
<i>M. simiae</i>	ATCC 25275 ^T	CBMJ00000000.2	21
<i>M. smegmatis</i>	ATCC 19420 ^T	LN831039.1	19.5
<i>M. thermoresistibile</i>	ATCC 19527 ^T	BCTB00000000.1	20.1
<i>M. triplex</i>	90-1019 ^T	CCAU00000000.1	21.5
<i>M. tuberculosis</i>	H37Rv ^T	NC_000962.3	21.5
<i>M. vaccae</i>	ATCC 15483 ^T	BCRS00000000.1	19.8
<i>M. vanbaalenii</i>	PYR-1 ^T	NC_008726.1	19.8
<i>M. vulneris</i>	NLA000700772 ^T	CCBG00000000.1	19.5
<i>M. yongonense</i>	05-1390 ^T	NC_021715.1	21.6
<i>Nocardia asteroides</i>	NBRC 15531 ^T	BAFO00000000.2	19.1

Date: 14 JUN 2017

Signature:



BEI Resources Authentication

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