

Mycobacterium alsense*, Strain TB 1906T*Catalog No. NR-49088**

Product Description: *Mycobacterium alsense* (*M. alsense*), strain TB 1906T was isolated in 2007 from the sputum of a 72-year-old patient with pulmonary disease in Denmark.

Lot¹: 64362405**Manufacturing Date:** 22JUL2016

| TEST | SPECIFICATIONS | RESULTS |
|-----------------------------------------------------------------------|------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Phenotypic Analysis^{2,3} | | |
| Cellular morphology | Rods | Rods |
| Colony morphology ⁴ | Report results | Circular, convex, entire, smooth and cream (Figure 1) |
| Growth rate | ≥ 7 days | 14 days |
| Growth at 45°C | Negative | Variable ⁵ |
| Growth at 55°C | Report results | Negative |
| Acid-fast stain | Positive (red colonies) | Positive (red colonies) |
| Pigmentation in the dark (Scotochromogen) | Positive | Negative (no pigment) ⁶ |
| Photoinduction for 1 hour (Photochromogen) | Negative (no pigment) | Negative (no pigment) |
| Nonchromogen (no pigment) | Negative | Positive ⁶ |
| Biochemical tests | | |
| Catalase | Report results | Positive |
| Catalase (semiquantitative) | Negative | Positive ⁷ |
| Catalase (68°C) | Positive | Positive |
| Iron uptake | Report results | Negative |
| Nitrate reduction | Negative | Negative |
| Tween 80 hydrolysis | Negative | Positive ⁸ |
| Urease | Negative | Positive ⁹ |
| Growth in the presence of 5% sodium chloride | Negative | Positive ¹⁰ |
| Growth in the presence of thiophene-2-carboxylic acid hydrazide (TCH) | Positive | Positive |
| Growth on MacConkey agar without crystal violet | Negative | Negative |
| Genotypic Analysis | | |
| Sequencing of 16S ribosomal RNA gene (~1540 base pairs) | ≥ 99% sequence identity to <i>M. alsense</i> type strain (GenBank: AJ938169) | 99% sequence identity to <i>M. alsense</i> type strain (GenBank: AJ938169) |
| Digital DNA-DNA hybridization (dDDH) ¹¹ | ≥ 70% for species identification | Not determined ^{12,13} (Table 1) |
| Purity (post-freeze) | | |
| Middlebrook 7H10 agar with OADC enrichment ¹⁴ | Growth consistent with expected colony morphology Report results | Growth consistent with expected colony morphology |
| Tryptic Soy agar ¹⁴ | Report results | Growth consistent with expected colony morphology |
| Viability (post-freeze)⁴ | Growth | Growth |

¹NR-49088 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment and grown for 7 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. alsense* was performed following: Tortoli, E., et al. "*Mycobacterium alsense* sp. nov., a Scotochromogenic Slow Grower Isolated from Clinical Respiratory Specimens." *Int. J. Syst. Evol. Microbiol.* 66 (2016): 450-456. PubMed: 26545358.

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

⁵NR-49088 was deposited as *M. alsense* and reported to be negative for growth at 42°C. Testing performed by BEI Resources indicates growth was observed after 14 days at 45°C in an aerobic atmosphere in Middlebrook 7H9 broth with ADC enrichment and after 21 days at 45°C in an aerobic atmosphere on Middlebrook 7H10 agar with OADC enrichment. Growth was not observed after 28 days at 45°C in an aerobic atmosphere on

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Lowenstein-Jensen agar.

⁶NR-49088 was deposited as *M. alsense* and reported to be positive for pigmentation in the dark (scotochromogen). Testing performed in triplicate by BEI Resources indicates NR-49071 tested positive as a nonchromogen.

⁷NR-49088 was deposited as *M. alsense* and reported to be negative for catalase (semiquantitative). Testing performed by BEI Resources indicates a positive result.

⁸NR-49088 was deposited as *M. alsense* and reported to be negative for Tween 80 hydrolysis. Testing performed by BEI Resources indicates a positive result.

⁹NR-49088 was deposited as *M. alsense* and reported to be negative for urease. Testing performed by BEI Resources indicates a positive result.

¹⁰NR-49088 was deposited as *M. alsense* and reported to be negative for growth in presence of 5% sodium chloride. Testing performed by BEI Resources indicates a positive result.

¹¹Relatedness 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.

¹²The whole genome of *M. alsense*, strain TB 1906T (Contig Total length ~ 11.5 megabase pairs) was sequenced using the Illumina® MiSeq® system and was assembled and analyzed with CLC Genomics Workbench Version 7.0.2.

¹³The 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.

¹⁴Purity of this lot was assessed for 14 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-49088 (Deposited as: <i>M. alsense</i>) |
|------------------------------------------------|----------------------------|-----------------|--------------------------------------------------------|
| <i>M. abscessus</i> subsp. <i>abscessus</i> | Hauduroy L948 ^T | NC_010397.1 | 19 |
| <i>M. abscessus</i> subsp. <i>bolletii</i> | BD ^T | AHAS00000000.1 | 18.9 |
| <i>M. abscessus</i> subsp. <i>massiliense</i> | CCUG 48898 ^T | NZ_AP014547.1 | 19 |
| <i>M. aromaticivorans</i> | JS19b1 ^T | JALN00000000.2 | 20 |
| <i>M. aurum</i> | ATCC 23366 ^T | CVQQ01000001.1 | 20.2 |
| <i>M. austroafricanum</i> | E9789-SA12441 ^T | HG964450.1 | 19.8 |
| <i>M. avium</i> subsp. <i>avium</i> | ATCC 25291 ^T | ACFI00000000.1 | 24.7 |
| <i>M. avium</i> subsp. <i>paratuberculosis</i> | ATCC 19698 ^T | AGAR00000000.1 | 25.2 |
| <i>M. avium</i> subsp. <i>silvaticum</i> | 6409 ^T | AYOC00000000.1 | 25.1 |
| <i>M. boemicum</i> | CIP 105808 ^T | CSTD01000001.1 | 24.7 |
| <i>M. canariensis</i> | 502329 ^T | BCSY00000000.1 | 20.1 |
| <i>M. celatum</i> | ATCC 51131 ^T | BBUN00000000.1 | 22.7 |
| <i>M. chelonae</i> | CM 6388 ^T | CP010946.1 | 19 |
| <i>M. chlorophenicolum</i> | PCP-I ^T | JYNL00000000.1 | 20.2 |
| <i>M. chubuense</i> | 48013 ^T | NC_018027.1 | 20.3 |
| <i>M. colombiense</i> | 10B ^T | AFVW00000000.2 | 24.1 |
| <i>M. conceptionense</i> | D16 ^T | CTEF00000000.1 | 20.1 |
| <i>M. cosmeticum</i> | LTA-388 ^T | CCBB000000000.1 | 20 |
| <i>M. crocinum</i> | czh-42 ^T | BBHD00000000.1 | 21.3 |
| <i>M. farcinogenes</i> | IEMVT 75 ^T | CCAY000000000.1 | 20 |

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| Species | Strain | Accession # | GGD vs. NR-49088 (Deposited as: <i>M. alsense</i>) |
|----------------------------------------------------|---------------------------|----------------|--------------------------------------------------------|
| <i>M. fluoranthenivorans</i> | FA4 ^T | BBFT00000000.1 | 21.2 |
| <i>M. fortuitum</i> subsp. <i>fortuitum</i> | ATCC 6841 ^T | CP014258.1 | 19.8 |
| <i>M. fortuitum</i> subsp. <i>acetamidolyticum</i> | NCH E11620 ^T | BCSZ00000000.1 | 19.7 |
| <i>M. gastri</i> | ATCC 15754 ^T | AZYN00000000.1 | 22.9 |
| <i>M. genavense</i> | 2289 ^T | JAGZ00000000.1 | 23.3 |
| <i>M. haemophilum</i> | ATCC 29548 ^T | CP011883.2 | 22.3 |
| <i>M. hassiacum</i> | 3849 ^T | ARBU00000000.1 | 20.4 |
| <i>M. hodleri</i> | EMI2 ^T | BBGO00000000.1 | 22.8 |
| <i>M. intracellulare</i> | ATCC 13950 ^T | NC_016946.1 | 24.3 |
| <i>M. kansasii</i> | ATCC 12478 ^T | NC_022663.1 | 22.7 |
| <i>M. kyorinense</i> | KUM 060204 ^T | BBKA00000000.1 | 22.1 |
| <i>M. mageritense</i> | 938 ^T | CCBF00000000.1 | 20.1 |
| <i>M. neoaurum</i> | ATCC 25795 ^T | JMDW00000000.1 | 19.4 |
| <i>M. neworleansense</i> | W6705 ^T | CWKH00000000.1 | 20 |
| <i>M. novocastrense</i> | 73 ^T | BCTA00000000.1 | 20.2 |
| <i>M. obuense</i> | 47001 ^T | JYNU00000000.1 | 20 |
| <i>M. pallens</i> | czh-8 ^T | BBHE00000000.1 | 21.5 |
| <i>M. parascrofulaceum</i> | HSC-68 ^T | ADNV00000000.1 | 24.9 |
| <i>M. pseudoshottsii</i> | L15 ^T | BCND00000000.1 | 21.6 |
| <i>M. pyrenivorans</i> | 17A3 ^T | BBHB00000000.1 | 21.9 |
| <i>M. rufum</i> | JS14 ^T | JROA00000000.1 | 20.1 |
| <i>M. rutilum</i> | czh-117 ^T | BBHF00000000.1 | 23.4 |
| <i>M. septicum</i> | W4964 ^T | CBMO00000000.1 | 20.2 |
| <i>M. setense</i> | ABO-M06 ^T | JTW00000000.1 | 19.9 |
| <i>M. simiae</i> | ATCC 25275 ^T | CBMJ00000000.2 | 22.7 |
| <i>M. smegmatis</i> | ATCC 19420 ^T | LN831039.1 | 20 |
| <i>M. thermoresistibile</i> | ATCC 19527 ^T | BCTB00000000.1 | 20.3 |
| <i>M. triplex</i> | 90-1019 ^T | CCAU00000000.1 | 23.6 |
| <i>M. tuberculosis</i> | H37Rv ^T | NC_000962.3 | 22.5 |
| <i>M. vaccae</i> | ATCC 15483 ^T | BCRS00000000.1 | 20.3 |
| <i>M. vanbaalenii</i> | PYR-1 ^T | NC_008726.1 | 20.1 |
| <i>M. vulneris</i> | NLA000700772 ^T | CCBG00000000.1 | 20.2 |
| <i>M. yongonense</i> | 05-1390 ^T | NC_021715.1 | 24.4 |
| <i>Nocardia asteroides</i> | NBRC 15531 ^T | BAFO00000000.2 | 19.4 |

Date: 20 JUL 2017

Signature:



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