# Certificate of Analysis for NR-49083

## *Mycobacterium longobardum*, Strain FI-07034T

## Catalog No. NR-49083

**Product Description:** *Mycobacterium longobardum* (*M. longobardum*), strain FI-07034T was isolated in 2006 from a 72-year-old female with bronco-pneumonitis in Lombardy, Italy.

**Lot[1]: 64362425** **Manufacturing Date: 02AUG2016**

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Phenotypic Analysis[2,3]** | | |
| Cellular morphology | Rods | Rods |
| Colony morphology[4] | Report results | Circular, low convex, entire, rough and cream (Figure 1) |
| Growth rate | ≥ 7 days | 11 days |
| Growth at 45°C | Negative | Positive[5] |
| Growth at 55°C | Report results | 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 | Positive |
| Biochemical tests | | |
| Catalase | Report results | Positive |
| Catalase (semiquantitative) | Positive | Positive |
| Catalase (68°C) | Positive | Positive |
| Iron uptake | Report results | Negative |
| Nitrate reduction | Positive | Negative[6] |
| Tween 80 hydrolysis | Negative | Positive[7] |
| Urease | Negative | Negative |
| Growth in the presence of 5% sodium chloride | Report results | Positive |
| Growth in the presence of thiophene-2-carboxylic acid hydrazide (TCH) | Positive | Positive |
| **Genotypic Analysis** | | |
| Sequencing of 16S ribosomal RNA gene (1500 base pairs) | ≥ 99% sequence identity to *M. longobardum* type strain (GenBank: JN571166.1) | 99.9% sequence identity to *M. longobardum* type strain[8] (GenBank: JN571166.1) |
| Digital DNA-DNA hybridization (dDDH)[9] | ≥ 70% for species identification | Not determined[10,11] (Table 1) |
| **Purity (post-freeze)** | | |
| Middlebrook 7H10 agar with OADC enrichment[12] | Growth consistent with expected colony morphology | Growth consistent with expected colony morphology |
| Tryptic Soy agar[12] | Report results | Growth consistent with expected colony morphology |
| **Viability (post-freeze)[4]** | Growth | Growth |

[1]NR-49083 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment. Broth inoculum was added to Middlebrook 7H10 agar with OADC enrichment. The inoculated agar and broth were each grown for 14 days at 37°C in an aerobic atmosphere with 5% $CO_2$. Colonies from both the Middlebrook 7H10 agar with OADC enrichment culture and the Middlebrook 7H9 broth with ADC enrichment culture were used to inoculate Middlebrook 7H10 agar with OADC enrichment kolles, which were grown for 7 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-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]Phenotypic characterization of *M. longobardum* 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 longobardum* sp. nov." Int. J. Syst. Evol. Microbiol. 63 (2013): 401-411. PubMed: 22447702.

[4]11 days at 37°C in an aerobic atmosphere with 5% $CO_2$ on Middlebrook 7H10 agar with OADC enrichment

[5]NR-49083 was deposited as *M. longobardum* and reported to be negative for growth at 45°C. Testing performed in triplicate by BEI Resources indicates a positive result.

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[6]NR-49083 was deposited as *M. longobardum* and reported to be positive for nitrate reduction. Testing performed in triplicate by BEI Resources indicates a negative result.
[7]NR-49083 was deposited as *M. longobardum* and reported to be negative for Tween 80 hydrolysis. Testing performed in triplicate by BEI Resources indicates a positive result.
[8]*M. longobardum*, strain FI-07034T is also referred to as strain DSM 45394 (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.).
[9]Relatedness 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.
[10]The whole genome of *M. longobardum*, strain FI-07034T (Contig Total Length ~ 4.5 megabase pairs) was sequenced using the Illumina® MiSeq® system and was assembled and analyzed with CLC Genomics Workbench Version 7.0.2.
[11]The 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.
[12]Purity of this lot was assessed for 11 days at 37°C in an aerobic atmosphere with 5% $CO_2$.

**Figure 1: Colony Morphology**

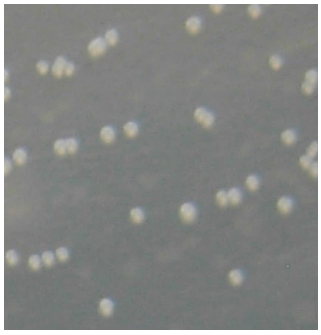

**Table 1: Digital DNA-DNA hybridization (dDDH)**

| Species | Strain | Accession # | GGD vs. NR-49083 (Deposited as: *M. longobardum*) |
|---|---|---|---|
| *M. abscessus* subsp. *abscessus* | Hauduroy L948$^T$ | NC_010397.1 | 20 |
| *M. abscessus* subsp. *bolletii* | BD$^T$ | AHAS00000000.1 | 19.8 |
| *M. abscessus* subsp. *massiliense* | CCUG 48898$^T$ | NZ_AP014547.1 | 19.5 |
| *M. aromaticivorans* | JS19b1$^T$ | JALN00000000.2 | 20 |
| *M. aurum* | ATCC 23366$^T$ | CVQQ01000001.1 | 19.6 |
| *M. austroafricanum* | E9789-SA12441$^T$ | HG964450.1 | 19.8 |
| *M. avium* subsp. *avium* | ATCC 25291$^T$ | ACFI00000000.1 | 21.2 |
| *M. avium* subsp. *paratuberculosis* | ATCC 19698$^T$ | AGAR00000000.1 | 21.7 |
| *M. avium* subsp. *silvaticum* | 6409$^T$ | AYOC00000000.1 | 21.7 |
| *M. bohemicum* | CIP 105808$^T$ | CSTD01000001.1 | 21.2 |
| *M. canariasense* | 502329$^T$ | BCSY00000000.1 | 20.2 |
| *M. celatum* | ATCC 51131$^T$ | BBUN00000000.1 | 21.6 |
| *M. chelonae* | CM 6388$^T$ | CP010946.1 | 19.6 |
| *M. chlorophenicolum* | PCP-I$^T$ | JYNL00000000.1 | 20.1 |
| *M. chubuense* | 48013$^T$ | NC_018027.1 | 20.2 |
| *M. colombiense* | 10B$^T$ | AFVW00000000.2 | 20.9 |
| *M. conceptionense* | D16$^T$ | CTEF00000000.1 | 20.5 |
| *M. cosmeticum* | LTA-388$^T$ | CCBB000000000.1 | 20.5 |
| *M. crocinum* | czh-42$^T$ | BBHD00000000.1 | 21.4 |
| *M. farcinogenes* | IEMVT 75$^T$ | CCAY000000000.1 | 20.1 |
| *M. fluoranthenivorans* | FA4$^T$ | BBFT00000000.1 | 21.5 |
| *M. fortuitum* subsp. *fortuitum* | ATCC 6841$^T$ | CP014258.1 | 20 |

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# Certificate of Analysis for NR-49083

| Species | Strain | Accession # | GGD vs. NR-49083 (Deposited as: *M. longobardum*) |
|---|---|---|---|
| *M. fortuitum* subsp. *acetamidolyticum* | NCH E11620$^T$ | BCSZ00000000.1 | 20.2 |
| *M. gastri* | ATCC 15754$^T$ | AZYN00000000.1 | 20.8 |
| *M. genavense* | 2289$^T$ | JAGZ00000000.1 | 20.5 |
| *M. haemophilum* | ATCC 29548$^T$ | CP011883.2 | 19.9 |
| *M. hassiacum* | 3849$^T$ | ARBU00000000.1 | 20.4 |
| *M. hodleri* | EMI2$^T$ | BBGO00000000.1 | 22.6 |
| *M. intracellulare* | ATCC 13950$^T$ | NC_016946.1 | 20.8 |
| *M. kansasii* | ATCC 12478$^T$ | NC_022663.1 | 20.4 |
| *M. kyorinense* | KUM 060204$^T$ | BBKA00000000.1 | 21 |
| *M. mageritense* | 938$^T$ | CCBF000000000.1 | 20.3 |
| *M. neoaurum* | ATCC 25795$^T$ | JMDW00000000.1 | 19.8 |
| *M. neworleansense* | W6705$^T$ | CWKH00000000.1 | 20.3 |
| *M. novocastrense* | 73$^T$ | BCTA00000000.1 | 20 |
| *M. obuense* | 47001$^T$ | JYNU00000000.1 | 19.9 |
| *M. pallens* | czh-8$^T$ | BBHE00000000.1 | 21.6 |
| *M. parascrofulaceum* | HSC-68$^T$ | ADNV00000000.1 | 21.2 |
| *M. pseudoshottsii* | L15$^T$ | BCND00000000.1 | 20.4 |
| *M. pyrenivorans* | 17A3$^T$ | BBHB00000000.1 | 21.7 |
| *M. rufum* | JS14$^T$ | JROA00000000.1 | 20.2 |
| *M. rutilum* | czh-117$^T$ | BBHF00000000.1 | 23.1 |
| *M. septicum* | W4964$^T$ | CBMO000000000.1 | 20.4 |
| *M. setense* | ABO-M06$^T$ | JTJW00000000.1 | 20.2 |
| *M. simiae* | ATCC 25275$^T$ | CBMJ000000000.2 | 20.3 |
| *M. smegmatis* | ATCC 19420$^T$ | LN831039.1 | 20.3 |
| *M. thermoresistibile* | ATCC 19527$^T$ | BCTB00000000.1 | 20.4 |
| *M. triplex* | 90-1019$^T$ | CCAU000000000.1 | 20.8 |
| *M. tuberculosis* | H37Rv$^T$ | NC_000962.3 | 20.5 |
| *M. vaccae* | ATCC 15483$^T$ | BCRS00000000.1 | 20.3 |
| *M. vanbaalenii* | PYR-1$^T$ | NC_008726.1 | 20.1 |
| *M. vulneris* | NLA000700772$^T$ | CCBG000000000.1 | 20.3 |
| *M. yongonense* | 05-1390$^T$ | NC_021715.1 | 21.2 |
| *Nocardia asteroides* | NBRC 15531$^T$ | BAFO00000000.2 | 19.6 |

**Date:** 16 JUN 2017 **Signature:**

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NR-49083_64362425_16JUN2017