Certificate of Analysis for NR-122

Mycobacterium tuberculosis subsp. *tuberculosis*, Strain H37Ra

Catalog No. NR-122

(Derived from ATCC® 25177™)

Product Description:

Mycobacterium tuberculosis (M. tuberculosis) subsp. *tuberculosis*, strain H37Ra is an attenuated strain derived from the virulent parent strain H37. Strain H37 was isolated in 1905 from the sputum of a patient with chronic pulmonary tuberculosis. NR-122 was produced by inoculation of BEI Resources seed lot 3685766 into Middlebrook 7H9 broth with ADC enrichment and grown for 21 days at 37°C in an aerobic atmosphere. Broth inoculum was added to Middlebrook 7H10 agar with OADC enrichment kolles and grown for 10 days at 37°C in an aerobic atmosphere to produce this lot.

Lot: 70069274 **Manufacturing Date: 25JUL2024**

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TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology 15 days at 37°C in an aerobic atmosphere on Middlebrook 7H10 agar with OADC enrichment	Report results	Irregular, low convex, undulate, rough and cream (Figure 1)
VITEK® MS (MALDI-TOF)	*M. tuberculosis* Complex	*M. tuberculosis* Complex (99.9%)
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1410 base pairs)	≥ 99% sequence identity to *M. tuberculosis*, strain H37Ra (GenBank: CP000611.1)	100% sequence identity to *M. tuberculosis,* strain H37Ra (GenBank: CP000611.1)[1]
Sequencing of Heat Shock Protein 65 gene (~ 440 base pairs)	≥ 99% sequence identity to *M. tuberculosis*, strain H37Ra (GenBank: CP000611.1)	100% sequence identity to *M. tuberculosis,* strain H37Ra (GenBank: CP000611.1)[1]
Digital DNA-DNA hybridization (dDDH)[2]	≥ 70% for species identification	*M. tuberculosis* (99.9%)[3] *M. microti* (98.8%) *M. caprae* (98.7%) *M. africanum* (98.6%) *M. pinnepedii* (98.3%) *M. bovis* (98.2%)
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment[4] 15 days at 37°C in an aerobic atmosphere	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Tryptic Soy agar 15 days at 37°C in an aerobic atmosphere with and without 5% CO_2	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze) 15 days at 37°C in an aerobic atmosphere on Middlebrook 7H10 agar with OADC enrichment	Growth	Growth

[1] Also consistent with other members of the *M. tuberculosis* complex

[2] 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.

[3] The whole genome of *M. tuberculosis* subsp. *tuberculosis*, strain H37Ra (contig total length approximately 7.34 megabase pairs) was sequenced using the Illumina® MiSeq® system and was analyzed and assembled with Kraken version 2.1.3 and Unicycler v0.5.0.

[4] Middlebrook 7H10 agar with OADC enrichment contains malachite green, which may inhibit growth of contaminating microorganisms.

Figure 1: Colony Morphology

/Sonia Bjorum Brower/
Sonia Bjorum Brower 28 JUL 2025
Technical Manager or designee, ATCC Federal Solutions

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