

Certificate of Analysis for NR-50276

Brucella melitensis, Strain 16M\(\Delta\vij\)bR

Catalog No. NR-50276

This reagent is the property of the U.S. Government.

Product Description: Brucella melitensis (B. melitensis), strain $16M\Delta vjbR$ is an attenuated vaccine strain that was adapted from B. melitensis, strain 16M.

Lot¹: 70006632 Manufacturing Date: 23JUN2017

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology ²	Report results	Gram-negative rods
Colony morphology ²	Report results	Circular, low convex, entire, smooth and cream (Figure 1)
Motility ³	Non-motile	Non-motile
Biochemical tests ⁴		
Oxidase	Positive	Positive
Urease	Positive (> 5 minutes)	Positive (> 5 minutes)
Hydrogen sulfide production (lead acetate)	Positive	Positive
Arginine dihydrolase	Negative	Negative
Arabinose	Negative	Positive ⁵
Glucose	Positive	Positive
Xylose	Positive ⁴	Positive
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene	≥ 99% sequence identity to	100% sequence identity to
(~ 1490 base pairs)	B. melitensis, strain 16M	B. melitensis, strain 16M
	(GenBank: CP034103)	(GenBank: CP034103)
Digital DNA-DNA hybridization (dDDH) ⁶	≥ 70% for species identification	B. melitensis (97.8%) ^{7,8}
Confirmation of deletion by Next-Generation	Δ <i>vjbR</i> (locus BMEII1116)	ΔvjbR (locus BMEII1116)9
Sequencing (illumina® MiSeq)		
Purity (post-freeze) ¹⁰	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze) ²	Growth	Growth

¹NR-50276 was produced by inoculation of the deposited material into Tryptic Soy broth and grown 2 days at 37°C in an aerobic atmosphere with 5% CO₂. Broth inoculum was added to Tryptic Soy agar with 5% defibrinated sheep blood agar kolles and grown 2 days at 37°C in an aerobic atmosphere with 5% CO₂ to produce this lot.

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

BEI Resources www.beiresources.org E-mail: contact@beiresources.org
Tel: 800-359-7370

Fax: 703-365-2898

²3 days at 37°C in an aerobic atmosphere on Tryptic Soy agar

³Motility test was performed on Remel™ Motility Test Medium w/TTC indicator for 3 days at 37°C in an aerobic atmosphere with 5% CO₂.

⁴Specifications are based on the testing results for the parent strain (BEI Resources NR-256) and may not agree with the expected specifications for *B. melitensis*.

⁵Arabinose metabolism was tested three times and was positive each time for this strain. This result differs from parental strain, and may be a result of the genetic manipulation of this strain or a spontaneous mutation.

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." <u>Stand. Genomic Sci.</u> 2 (2010): 117-134. PubMed: 21304684.

⁷The whole genome of *B. melitensis*, strain 16MΔ*vjbR* (Genome Total Length 3,270,279 base pairs) was sequenced using the Illumina[®] MiSeq[®] system and was assembled and analyzed with CLC Genomics Workbench Version 7.0.2.

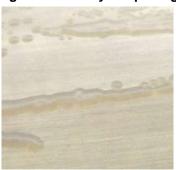
⁸The dDDH testing was not able to distinguish between *Brucella* spp. (abortus, canis, ceti, inopinata, melitensis, microti, neotomae, ovis, pinnipedialis, suis and vulpis). All of these species produced scores >70% and thus, by the dDDH criteria, are considered the same species.

⁹Alignment of the NR-50276 DNA sequence to the strain 16M sequence illustrates the 760 base pair deletion described by the contributor (Arenas-Gamboa, A. M., et al. "Extended Safety and Efficacy Studies of the Attenuated *Brucella* Vaccine Candidates 16MΔ*vjbR* and S19Δ*vjbR* in the Immunocompromised IRF-1." Mouse Model." Clin. Vaccine Immunol. 19 (2012): 249-260. PubMed: 22169089.).



Certificate of Analysis for NR-50276

Figure 1: Colony Morphology



/Heather Couch/ Heather Couch

19 MAR 2019

Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

BEI Resources www.beiresources.org E-mail: contact@beiresources.org
Tel: 800-359-7370

Fax: 703-365-2898