

Certificate of Analysis for NR-9955

Genomic DNA from Brucella suis, Strain Thomsen

Catalog No. NR-9955

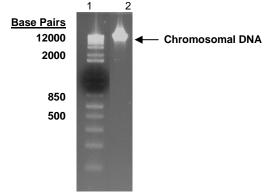
Product Description: Genomic DNA was extracted from a preparation of *Brucella suis* (*B. suis*), strain Thomsen, biovar 2. *B. suis*, strain Thomsen was isolated in 1951 from a hare in Denmark and deposited to the ATCC[®] in 1967. Note: The label on the vial is incorrect; NR-9955 was extracted from ATCC[®] 23445[™] not NR-303.

Lot¹: 64364110 Manufacturing Date: 24JUN2016

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1360 base pairs) Digital DNA-DNA hybridization (dDDH) ³	≥ 99% sequence identity to <i>B. suis</i> , strain Thomsen (GenBank: CP000912.1) ≥ 70% for species identification	100% sequence identity to <i>B. suis</i> , strain Thomsen (GenBank: CP000912.1) ² 98.7% <i>B. suis</i> ⁴
Agarose Gel Electrophoresis	High molecular weight chromosomal DNA	High molecular weight chromosomal DNA (Figure 1)
Concentration by PicoGreen® Measurement	0.7 to 1.5 µg in 25 to 100 µL per vial	1.1 µg in 29 µL per vial (36 µg/mL)
Amount per vial	0.7 to 1.5 μg	1.1 µg
Functional Activity by PCR Amplification 16S ribosomal RNA gene	~ 1500 base pair amplicon	~ 1500 base pair amplicon
OD ₂₆₀ /OD ₂₈₀ Ratio	1.7 to 2.1	1.9
Bacterial Inactivation 10% of total yield plated on agar for 14 days ⁵	No viable bacteria detected	No viable bacteria detected

¹The bacterial preparation used for extraction of genomic DNA was produced from a culture of ATCC[®] 23445[™] lot 45199. Genomic DNA was extracted using proprietary technology.

Figure 1: Agarose Gel Electrophoresis



Lane 1: Invitrogen™ TrackIt 1 Kb Plus DNA Ladder™

Lane 2: ~ 200 ng of NR-9955

BEI Resources

www.beiresources.org

E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898

²Also consistent with other *Brucella* species and *Ochrobactrum* species; For more information, please see Velasco, J., et al. "Evaluation of the Relatedness of *Brucella* spp. and *Ochrobactrum anthropi* and Description of *Ochrobactrum intermedium* sp. nov., a New Species with a Closer Relationship to *Brucella* spp." Int. J. Syst. Bacteriol. 48 (1998): 759-768. PubMed: 9734029.

³Relatedness 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.

⁴B. abortus, B. canis, B. ceti, B. melitensis, B. microti, B. neotomae, B. ovis and B. pinnipedialis all had dDDH scores over 96% and B. inopinata and B. vulpis had scores of 81.5 and 80.7, respectively, indicating that dDDH analysis cannot differentiate the Brucella genus.

⁵An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-negative and Gram-positive bacteria.



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/Heather Couch/ Heather Couch

19 SEP 2018

Program Manager or designee, ATCC Federal Solutions

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Tel: 800-359-7370 Fax: 703-365-2898