

## Certificate of Analysis for NR-50370

### Genomic DNA from Brucella abortus, Strain 544

#### Catalog No. NR-50370

### **Product Description:**

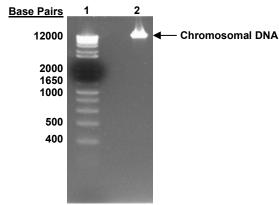
Genomic DNA was isolated from a preparation of Brucella abortus (B. abortus), strain 544 (NCTC 10093, ATCC® 23448™), biovar 1. B. abortus, strain 544 was isolated in 1936 from a bovine source in England.

Lot: 64375860<sup>1</sup> Manufacturing Date: 12AUG2016

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1430 base pairs)	≥ 99% sequence identity to <i>B. abortus</i> , strain 544 (GenBank: AQIS01000009.1)	100% sequence identity to <i>B. abortus,</i> strain 544 (GenBank: AQIS01000009.1) <sup>2</sup>
Digital DNA-DNA hybridization (dDDH) <sup>3</sup>	≥ 70% for species identification	99.4% B. abortus <sup>4,5</sup>
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 47 μL per vial (24 μg/mL)
Amount per vial	0.7 to 1.5 μg	1.1 µg
OD <sub>260</sub> /OD <sub>280</sub> Ratio	1.7 to 2.1	1.8
Bacterial Inactivation 10% of total yield plated on agar for 14 days <sup>6</sup>	No viable bacteria detected	No viable bacteria detected

¹The bacterial preparation used for extraction of genomic DNA was produced from a culture of ATCC® 23448™ lot 45214. 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-50370

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<sup>&</sup>lt;sup>2</sup>Also 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.

<sup>&</sup>lt;sup>3</sup>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.

<sup>&</sup>lt;sup>4</sup>The whole genome of *B. abortus*, strain 544 (∼ 3.27 megabase pairs) was sequenced using the Illumina® MiSeq® system and was assembled and analyzed using CLC Genomics Workbench Version 7.0.2.

<sup>&</sup>lt;sup>5</sup>B. canis, B. ceti, B. melitensis, B. microti, B. neotomae, B. ovis, B. pinnipedialis and B. suis all had dDDH scores over 96% and B. inopinata and B. vulpis had scores of 81% and 80.3%, respectively, indicating that dDDH analysis cannot differentiate the Brucella genus.

<sup>&</sup>lt;sup>6</sup>An 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 18 JUL 2019

Program Manager or designee, ATCC Federal Solutions

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