

Certificate of Analysis for NR-13362

Genomic DNA from Francisella tularensis subsp. novicida, Strain ∆IgID

Catalog No. NR-13362

Product Description: Genomic DNA was isolated from a preparation of *Francisella tularensis* (F. tularensis) subsp. novicida, strain $\Delta IgID$. F. tularensis subsp. novicida, strain $\Delta IgID$ is a transposon mutant of the wild-type strain U112, in which the igID gene region has been replaced with a mini-Tn5 insert, rendering it resistant to kanamycin.

Lot¹: 59882648 Manufacturing Date: 11APR2011

TEST	SPECIFICATIONS	RESULTS
Sequencing of 16S Ribosomal RNA Gene (~ 1370 base pairs)	Identical to BEI Resources NR-9713 Consistent with <i>F. tularensis</i>	Identical to BEI Resources NR-9713 Consistent with <i>F. tularensis</i>
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	0.9 μg in 32 μL per vial (28 μg/mL)
Functional Activity by PCR Amplification 16S ribosomal RNA gene	~ 1500 bp amplicon	~ 1500 bp amplicon
Molecular Subtyping by PCR Amplification of Subspecies-Specific Sequence from Extracted DNA ^{2,3}	~ 1500 bp amplicon (subsp. <i>tularensis</i>) ~ 900 bp amplicon (subsp. <i>holarctica</i>) ~ 3300 bp amplicon (subsp. <i>novicida</i>)	~ 3300 bp amplicon (subsp. <i>novicida</i>)
OD ₂₆₀ /OD ₂₈₀ Ratio	1.7 to 2.0	2.0
Bacterial Inactivation 10% of total yield plated on Chocolate Agar ^{4,5}	No viable bacteria detected	No viable bacteria detected

¹NR-13362 was produced by inoculation of NR-9713 (Lot 58893070) into Brain Heart Infusion Broth and grown 24 hours at 37°C. Broth inoculum was added to Chocolate agar Kolles which were grown 24 hours at 37°C and genomic DNA was extracted using proprietary technology.

Date: 11 NOV 2011

Signature:

Title: Technical Manager, BEI Authentication or designee

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.

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²Broekhuijsen, M., et al. "Genome-Wide DNA Microarray Analysis of *Francisella tularensis* Strains Demonstrates Extensive Genetic Conservation within the Species but Identifies Regions that are Unique to the Highly Virulent *F. tularensis* subsp. *tularensis*." <u>J. Clin. Microbiol.</u> 41 (2003): 2924-2931. PubMed: 12843022.

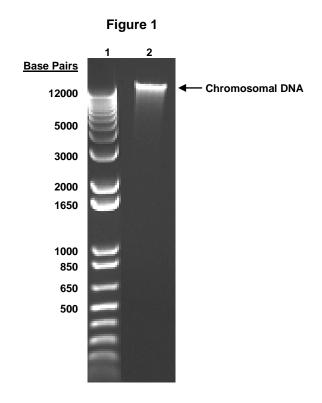
³The Molecular Subtyping by PCR Amplification of Subspecies-Specific Sequence from Extracted DNA was performed on NR-13362 lot 58893072 which was extracted from the same material that was used to produce NR-9713 lot 58893070. NR-13362 lot 59882648 was extracted from NR-9713 lot 58893070.

⁴Incubated for 7 days at 37°C and aerobic atmosphere

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

SUPPORTING INFECTIOUS DISEASE RESEARCH

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

Lane 2: 200 ng of HM-13362

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