

Bacillus anthracis (Exempted), Strain ISP3191 (Deposited as *Bacillus cereus*, Strain ISP3191)

Catalog No. NR-22161

Product Description:

Bacillus anthracis (*B. anthracis*) (Exempted), strain ISP3191 was isolated in 2007 from a spice sample collected in Belgium. NR-22161 was deposited to BEI Resources as *Bacillus cereus*; however, digital DNA-DNA hybridization (dDDH) analysis performed at BEI Resources resulted in reclassification to *Bacillus anthracis*. Note: NR-22161 lacks the plasmids pXO1 and pXO2 and is acapsular, and is therefore exempted from select status. The deposited material was inoculated into Tryptic Soy broth and grown for 1 day at 30°C in an aerobic atmosphere, and the resulting subculture was vialled and frozen. NR-22161 was produced by inoculation of the frozen subculture into Tryptic Soy broth and grown for 1 day at 30°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar with 5% defibrinated sheep blood kolles, which were grown for 1 day at 30°C in an aerobic atmosphere to produce this lot. Quality control testing was completed under propagation conditions unless otherwise noted.

Lot: 63817644

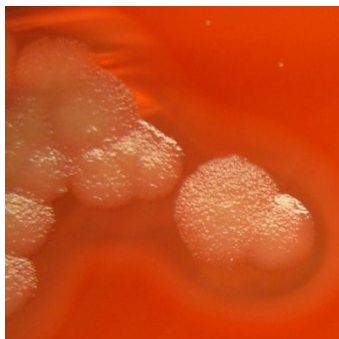
Manufacturing Date: 06NOV2015

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TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis¹ Cellular morphology Colony morphology Hemolysis Motility ³ BD BBL™ Motility Test Medium with TTC Indicator for 1 day at 37°C in an aerobic atmosphere Biochemical tests Production of acid from trehalose ⁵ Production of acid from salicin ⁵ Production of acid from glycerol ⁵ Nitrate reduction Arginine decarboxylase activity VITEK® MS (MALDI-TOF)	Gram-positive rods Report results Non-hemolytic Non-motile Report results Report results Report results Report results Report results <i>Bacillus</i> spp.	Gram-positive rods Circular, raised, undulate, rough and tan (Figure 1) β-hemolytic ² Motile ⁴ Positive Negative Negative Positive Negative <i>Bacillus</i> spp. (99.9%) ⁶
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1430 base pairs) Digital DNA-DNA hybridization (dDDH) ^{8,9}	≥ 99% sequence identity to <i>B. anthracis</i> , strain ISP3191 (GenBank: JH792131) ≥ 70% for species identification	99.7% sequence identity to <i>B. anthracis</i> , strain ISP3191 (GenBank: JH792131) ⁷ <i>B. anthracis</i> (80%)
PCR Amplification (in silico) of <i>B. anthracis</i> specific chromosomal region^{10,11}	Amplicon present	Amplicon present
Presence of Virulence Plasmids^{10,12} pXO1 (four targets) pXO2 (three targets)	Not present Not present	Not present ORF7 detected ¹³
Purity (post-freeze) 7 days at 30°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze)	Growth	Growth

- ¹Presumptive identification of *B. anthracis* was performed using phenotypic tests that eliminate other *B. cereus* group members (*B. cereus*, *B. thuringiensis* and *B. mycoides*).
- ²The non-hemolytic nature of *B. anthracis* has been used to distinguish it from other *Bacillus* sp., however, there are reports of *B. anthracis* strains that are hemolytic, as NR-22161 appears to be [Brown, E. R., et al. "Differential Diagnosis of *Bacillus cereus*, *Bacillus anthracis*, and *Bacillus cereus* var. *mycoides*." *J. Bacteriol.* 75 (1958): 499-509. PubMed: 13538915].
- ³In the *B. cereus* group, *B. cereus* and *B. thuringiensis* are motile, whereas *B. anthracis* and *B. mycoides* are non-motile.
- ⁴Specification for this test was obtained from Logan, N. A. and P. De Vos. "Family I. *Bacillaceae*." *Bergey's® Manual of Systematic Bacteriology, Second Edition, Volume Three*. (2009) De Vos, P., et al. (Ed.), Springer, which indicates that most strains of *B. anthracis* are non-motile; however, up to 15% of strains may be motile.
- ⁵Negative tests are observed for >7 days.
- ⁶The VITEK® MS (MALDI-TOF) not confirm the species of this organism but could confirm the genus. Results included *B. cereus* (33.3%), *B. mycoides* (33.3%) and *B. thuringiensis* (33.3%).
- ⁷*B. cereus* group species cannot be classified based on 16S sequence [Spencer, R. C. "*Bacillus anthracis*." *J. Clin. Pathol.* 56 (2003): 182-187. PubMed: 12610093].
- ⁸Relatedness 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.
- ⁹dDDH was completed against type strain genomes for *B. anthracis* and *B. cereus*. The results obtained were also published by Liu, Y., et al. "Genomic insights into the taxonomic status of the *Bacillus cereus* group." *Sci. Rep.* 5 (2015): 14082. PubMed: 26373441.
- ¹⁰For PCR primers used in these assays, refer to Riojas, M. A., et al. "Multiplex PCR for Species-Level Identification of *Bacillus anthracis* and Detection of pXO1, pXO2, and Related Plasmids." *Health Secur.* 13 (2015): 122-129. PubMed: 25813976.
- ¹¹This product was verified to a species level using a digital PCR-based assay to a *B. anthracis*-specific genetic mutation capable of differentiating *B. anthracis* from the remainder of the *B. cereus* group.
- ¹²Plasmid presence was verified using an *in silico* PCR-based assay to *B. anthracis*-plasmids pXO1 and pXO2.
- ¹³An ORF7 gene with homology to pXO2 was present, suggesting the presence of a pXO2-like plasmid. However, the plasmid does not contain the capsule genes.

Figure 1: Colony Morphology



/Sonia Bjorum Brower/

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Technical Manager or designee, ATCC Federal Solutions

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