SUPPORTING INFECTIOUS DISEASE RESEARCH

Genomic DNA from Bacillus anthracis, Strain Sterne BA723 (Alef243)

Catalog No. NR-9540

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

Product Description: Genomic DNA was isolated from a preparation of *Bacillus anthracis* (*B. anthracis*), strain Sterne BA723 (Δ *lef243*). Note: The designation BA723 refers to the numbering system used in the Stibitz laboratory.

Lot¹: 58109378

Manufacturing Date: 27FEB2008

TEST	SPECIFICATIONS	RESULTS
Sequencing of 16S Ribosomal RNA Gene (~ 1380 bp)	Identical to BEI Resources NR-9397 Consistent with <i>B. cereus</i> group ²	Identical to BEI Resources NR-9397 Consistent with <i>B. cereus</i> group ²
Presence or Absence of Plasmids Confirmed by PCR Amplification pXO1 pXO2	Positive Negative	Positive Negative
Agarose Gel Electrophoresis	High molecular weight chromosomal DNA	High molecular weight chromosomal DNA (Figure 1)
Content by PicoGreen [®] Measurement	4 to 6 μ g in 25 to 100 μ L per vial	5.4 μg in 45 μL per vial (121 μg/mL)
PCR Assay of Extracted DNA 16S ribosomal RNA gene Specific chromosomal marker ³ Presence of virulence plasmids ⁴ pXO1 (three targets) pXO2 (three targets) Verification of Δlef	~ 1500 bp amplicon; ~ 555 bp amplicon Amplicon present Amplicons present No amplicons No amplicon	~ 1500 bp amplicon; ~ 555 bp amplicon Amplicon present Amplicons present No amplicons No amplicon
OD ₂₆₀ /OD ₂₈₀ Ratio	1.7 to 1.9	1.8
Bacterial Inactivation 10% of total yield plated on Tryptic Soy Agar with 5% sheep blood ^{5,6}	No viable bacteria detected	No viable bacteria detected

¹B. anthracis, strain BA723 was deposited by E. Scott Stibitz, Division of Bacterial, Parasitic, and Allergenic Products, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland. The bacterial preparation used for extraction of genomic DNA was produced by Tryptic Soy Broth culture of the deposited material. After incubation for 24 hours at 35°C and aerobic atmosphere, genomic DNA was extracted using proprietary technology.

²Bacillus cereus group species (*B. cereus, B. thuringiensis, B. mycoides,* and *B. anthracis*) cannot be classified based on 16S sequence (Spencer, R. C. "Bacillus anthracis." J. Clin. Pathol. 56 (2003): 182-187. PubMed: 12610093).

³This product was verified to a species level using a proprietary (Patent Pending) PCR-based assay to a *Bacillus anthracis*-specific genetic mutation capable of differentiating *B. anthracis* from the remainder of the *B. cereus* group.

⁴Plasmids were verified using a proprietary (Patent Pending) PCR-based assay to a *Bacillus anthracis*-plasmids pXO1 and pXO2. ⁵7 days at 37°C in an aerobic atmosphere

⁶An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-positive and Gram-negative bacteria.

Date: 05 SEP 2012

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.

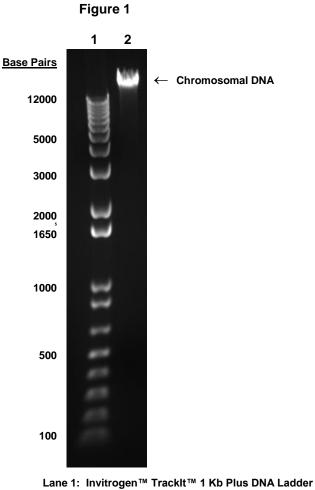
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Certificate of Analysis for NR-9540

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Lane 2: 200 ng of NR-9540