

Certificate of Analysis for NR-10300

Genomic DNA from Bacillus anthracis, Strain Sterne \(\Delta GBAA0552-2 \)

Catalog No. NR-10300

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 Δ GBAA0552-2, a deletion mutant of the toxigenic acapsulate original Sterne strain (34F2).

Lot¹: 59466951 Manufacturing Date: 26JAN2011

TEST	SPECIFICATIONS	RESULTS
Sequencing of 16S Ribosomal RNA Gene (~ 1460 bp)	Consistent with B. cereus group	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	0.7 to 1.5 μg in 25 to 100 μL per vial	0.8 μg in 31 μL per vial (25 μg/mL)
PCR Assay of Extracted DNA 16S ribosomal RNA gene Specific chromosomal marker³ Presence of virulence plasmids⁴ pXO1 (three targets) pXO2 (three targets) Verification of Δpag	~ 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 2.0	1.9
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

B. anthracis, strain Sterne \(\Delta \text{BAA0552-2}\) was deposited by Professor Philip Hanna, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan. The bacterial preparation used for extraction of genomic DNA was produced by Tryptic Soy Broth culture of BEI Resources NR-9996 (Lot 58394755). After incubation for 24 hours at 37°C in an aerobic atmosphere, genomic DNA was extracted using proprietary technology.

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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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.

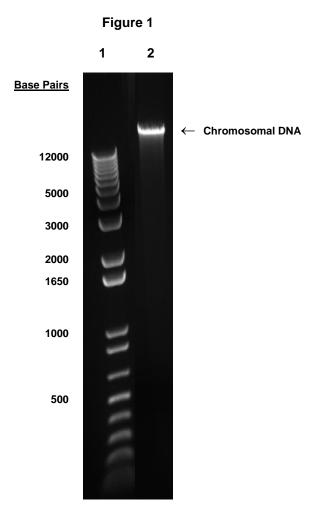
⁴Plasmids were verified using a proprietary (Patent Pending) PCR-based assay to a *Bacillus anthracis*-plasmids pXO1 and pXO2.

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.



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

Lane 2: 200 ng of NR-10300

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