

## **Certificate of Analysis for NR-9991**

## Bacillus anthracis, Strain Sterne 34F2, BA853 (∆asbC)

## Catalog No. NR-9991

**Product Description:** Bacillus anthracis (B. anthracis), strain Sterne 34F2, BA853 ( $\triangle asbC$ ) was deposited as a markerless, nonpolar, 1032 base pair deletion mutant of asbC, a member of the petrobactin biosynthetic operon (asbABCDEF).

Lot<sup>1</sup>: 58394746 Manufacturing Date: 12NOV2008

TEST	SPECIFICATIONS	RESULTS
1-41	6. 2616.11.6.16	
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology <sup>2</sup>	Report results	Circular, low convex, entire, ground- glass, opaque and gray (Figure 1)
Sporulation	Report results	No spores observed
Hemolysis <sup>2</sup>	Non-hemolytic	Non-hemolytic
Motility	Non-motile	Non-motile
Capsule (India ink staining)	Negative	Negative
Tenacious	Positive	Positive
Analytical profile index (API <sup>®</sup> 50 CHB including API <sup>®</sup> 20E; ONPG to GEL only)	B. anthracis (≥ 80%)	B. anthracis (38.6%) <sup>3</sup>
Nitrate reduction	Positive	Positive
Genotypic Analysis Sequencing of 16S ribosomal RNA (rRNA) gene (~ 690 base pairs)	> 99% identical to <i>B. anthracis</i> , strain Sterne (GenBank: AE017225)	100% identical to <i>B. anthracis</i> , strain Sterne (GenBank: AE017225) <sup>4</sup>
PCR Amplification of <i>B. anthracis</i> specific chromosomal region <sup>5</sup>	~ 200 base pair amplicon	~ 200 base pair amplicon
Bacillus anthracis specific prophage PCR <sup>6</sup>		
16S rRNA gene	Amplicon present	Amplicon present
Prophage 1	Report results	No amplicon present
Prophage 2	Report results	Amplicon present
Prophage 3	Report results	No amplicon present
Prophage 4	Report results	Amplicon present
Presence of Plasmids Confirmed by PCR Amplification <sup>7,8</sup>		
16S rRNA gene	Amplicon present	Amplicon present
pXO1 (four targets)	Amplicons present	Amplicons present
pXO2 (three targets)	No amplicons	No amplicons
Viability (post-freeze) <sup>2</sup>	Growth	Growth

NR-9991 was produced by inoculation of the deposited material into Brain Heart Infusion broth and grown 1 day at 30°C in an aerobic atmosphere. 10% glycerol was added to the resulting growth and the mixture was cryopreserved. A vial of the preserved material was thawed and used to inoculate a tube of Tryptic Soy broth and grown 1 day at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar with 5% defibrinated sheep blood kolles which were grown 1 day at 37°C in an aerobic atmosphere to produce this lot.

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<sup>&</sup>lt;sup>2</sup>1 day at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood

<sup>&</sup>lt;sup>3</sup>99.8% probability that the genus is Bacillus; 38.6% B. anthracis, 34% B. mycoides and 27.2% B. cereus.

<sup>&</sup>lt;sup>4</sup>Also consistent with *B. cereus* group species (*B. cereus, B. thuringiensis, B. mycoide*s, and *B. anthracis*) which cannot be classified based on 16S sequence (Spencer, R. C. "*Bacillus anthracis*." J. Clin. Pathol. 56 (2003): 182-187. PubMed: 12610093).

<sup>&</sup>lt;sup>5</sup>This product was verified to a species level using a PCR-based assay to a *B. anthracis*-specific genetic mutation capable of differentiating *B. anthracis* from the remainder of the *B. cereus* group.

<sup>&</sup>lt;sup>6</sup>The prophage-specific multiplex PCR detects 4 prophages that only *B. anthracis* contains, no other species of *Bacillus* has been known to contain more than one prophage (Sozhamannan, S., et al. "The *Bacillus anthracis* Chromosome Contains Four Conserved, Excision-Proficient, Putative Prophages." <u>BMC Microbiol</u>. 6 (2006): 34. PubMed: 16600039).

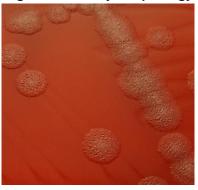


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<sup>7</sup>For PCR primers used in these assays, refer to Riojas, M. A., et al. "Multiplex PCR for Species-Level Identification of *Bacillus anthracis* and Detection of pXO1, pXO2, and Related Plasmids." <u>Health Security</u> 13 (2015): 122-129. PubMed: 25813976.

<sup>8</sup>Plasmids were verified using a PCR-based assay to *B. anthracis*-plasmids pXO1 and pXO2.

Figure 1: Colony Morphology



Date: 18 JAN 2017 Signature:

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