

**Bacillus anthracis, Strain Sterne 34F2, BA853 ( $\Delta asbC$ )**

**Catalog No. NR-9991**

**Product Description:** *Bacillus anthracis* (*B. anthracis*), strain Sterne 34F2, BA853 ( $\Delta asbC$ ) 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
<b>Phenotypic Analysis</b> Cellular morphology Colony morphology <sup>2</sup>  Sporulation Hemolysis <sup>2</sup> Motility Capsule (India ink staining) Tenacious Analytical profile index (API <sup>®</sup> 50 CHB including API <sup>®</sup> 20E; ONPG to GEL only) Nitrate reduction	Gram-positive rods Report results  Report results Non-hemolytic Non-motile Negative Positive <i>B. anthracis</i> ( $\geq 80\%$ )  Positive	Gram-positive rods Circular, low convex, entire, ground-glass, opaque and gray (Figure 1) No spores observed Non-hemolytic Non-motile Negative Positive <i>B. anthracis</i> (38.6%) <sup>3</sup>  Positive
<b>Genotypic Analysis</b> 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>
<b>PCR Amplification of <i>B. anthracis</i> specific chromosomal region<sup>5</sup></b>	~ 200 base pair amplicon	~ 200 base pair amplicon
<b><i>Bacillus anthracis</i> specific prophage PCR<sup>6</sup></b> 16S rRNA gene Prophage 1 Prophage 2 Prophage 3 Prophage 4	Amplicon present Report results Report results Report results Report results	Amplicon present No amplicon present Amplicon present No amplicon present Amplicon present
<b>Presence of Plasmids Confirmed by PCR Amplification<sup>7,8</sup></b> 16S rRNA gene pXO1 (four targets) pXO2 (three targets)	Amplicon present Amplicons present No amplicons	Amplicon present Amplicons present No amplicons
<b>Viability (post-freeze)<sup>2</sup></b>	Growth	Growth

<sup>1</sup>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.

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

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

<sup>4</sup>Also consistent with *B. cereus* group species (*B. cereus*, *B. thuringiensis*, *B. mycoides*, 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>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>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." *BMC Microbiol.* 6 (2006): 34. PubMed: 16600039).

<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." *Health Security* 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:

A handwritten signature in black ink, which appears to read "David C. Archer".

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