

## **Certificate of Analysis for NR-9997**

## Bacillus anthracis, Strain Sterne 34F2, ∆GBAA1346-2

## Catalog No. NR-9997

**Product Description:** Bacillus anthracis (B. anthracis), strain Sterne 34F2, ∆GBAA1346-2 was deposited as a putative internalin, null mutant.

Lot<sup>1</sup>: 58394756 Manufacturing Date: 12NOV2008

TEST	SPECIFICATIONS	RESULTS
1E31	SPECIFICATIONS	RESULIS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology <sup>2</sup>	Report results	Circular, flat, entire, ground-glass, opaque and gray (Figure 1)
Sporulation	Report results	Endospores present
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® 50 CHB including API® 20E; ONPG to GEL only)	B. anthracis (≥ 80%)	B. anthracis (81.3%)
Nitrate reduction	Positive	Positive
Genotypic Analysis Sequencing of 16S ribosomal RNA (rRNA) gene (~ 1400 base pairs)	> 99% identical to <i>B. anthracis</i> , strain Sterne (GenBank: AE017225)	99.9% identical to <i>B. anthracis</i> , strain Sterne (GenBank: AE017225) <sup>3</sup>
PCR Amplification of <i>B. anthracis</i> specific chromosomal region <sup>4</sup>	~ 200 base pair amplicon	~ 200 base pair amplicon
Presence of Plasmids Confirmed by PCR Amplification <sup>5,6</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-9997 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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www.beiresources.org

E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898

<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>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*." <u>J. Clin. Pathol.</u> 56 (2003): 182-187. PubMed: 12610093).

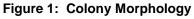
<sup>&</sup>lt;sup>4</sup>This product was verified to a species level using a PCR-based assay to a *B. anthracis*-specific genetic mutation capable of differentiating *B. anthracis* from the remainder of the *B. cereus* group.

<sup>&</sup>lt;sup>5</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>&</sup>lt;sup>6</sup>Plasmids were verified using a PCR-based assay to *B. anthracis*-plasmids pXO1 and pXO2.



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**Date:** 01 MAR 2017

Signature:

**BEI Resources Authentication** 

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