

## **Certificate of Analysis for NR-9993**

## Bacillus anthracis, Strain Sterne BA855 (∆asbE)

## Catalog No. NR-9993

**Product Description:** Bacillus anthracis (B. anthracis), strain Sterne BA855 is a markerless, nonpolar, 945 bp deletion mutant of the petrobactin biosynthetic gene ( $\triangle asbE$ ) of the toxigenic acapsulate original Sterne strain (34F2).

Lot<sup>1</sup>: 58394749 Manufacturing Date: 07NOV2008

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rod	Gram-positive rod
Colony morphology		
Tryptic Soy Agar, 5% sheep blood <sup>2</sup>	Report results	Circular, flat, erose, ground-glass,
23		opaque and grey (Figure 1)
PLET Agar <sup>2,3</sup>	Report results	Circular, flat, lobate, ground-glass,
Chamilation	Desition	opaque and cream (Figure 2)
Sporulation	Positive	Positive
Motility	Non-motile	Non-motile
β-hemolysis	Non-hemolytic	Non-hemolytic
Capsule (India ink staining)	Negative	Negative
Tenacious	Positive	Positive
Analytical profile index (API® 50 CHB	Consistent with B. anthracis	Consistent with B. anthracis
including API® 20E; ONPG to GEL only)	Positive	Positive
Nitrate reduction	Positive	Positive
Genotypic Analysis		,
Sequencing of 16S ribosomal RNA gene	Consistent with Bacillus cereus group	Consistent with Bacillus cereus group <sup>4</sup>
(1420 base pairs)		
PCR Assay of Extracted DNA		
16S ribosomal RNA gene	~ 1500 bp amplicon	~ 1500 bp amplicon
Presence of virulence plasmids	r r r	r r
pXO1 (aat)	~ 125 bp amplicon	~ 125 bp amplicon
pXO2 (at, capA, capB, capC)	No amplicons	No amplicons
Viability (post-vialing) <sup>5</sup>	Growth	Growth

<sup>&</sup>lt;sup>1</sup>B. anthracis, strain Sterne BA851 (ΔasbA) was deposited by Philip C. Hanna, Associate Professor, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan. NR-9993 was produced by inoculation of the deposited material into Tryptic Soy Broth and grown 24 hours at 37°C. Broth inoculum was added to Kolles which were grown 24 hours at 37°C to produce this lot.
<sup>2</sup>24 hours at 37°C

<sup>5</sup>24 hours at 37°C in Tryptic Soy Broth

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<sup>&</sup>lt;sup>3</sup>Growth on PLET [polymyxin-lysozyme-EDTA-thallous acetate] Agar (Hardy Diagnostics, Cat. No. G153) differentiates *B. anthracis* from other *Bacillus* species, including *B. cereus*, *B. thuringiensis* and *B. mycoides*, whose growth is inhibited by the combination of EDTA and thallium cations. Dragon, D. C. and R. P. Rennie. "Evaluation of Spore Extraction and Purification Methods for Selective Recovery of Viable *Bacillus anthracis* Spores." <u>Lett. Appl. Microbiol.</u> 33 (2001): 100-105. PubMed: 11472515.

<sup>&</sup>lt;sup>4</sup>Bacillus 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).



## **Certificate of Analysis for NR-9993**

Figure 1

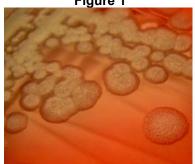
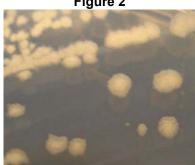


Figure 2



Date: 23 JUN 2009 Signature: Signature on File

> Title: Technical Manager, BEI Authentication or designee

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