

Bacillus anthracis, Strain Sterne BA695 (Δ cya244)

Catalog No. NR-9398

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Product Description: *Bacillus anthracis* (*B. anthracis*), strain Sterne BA695 (Δ cya244) is a 2,397 base pair deletion mutant of the toxigenic acapsulate Sterne 7702 strain. The designation BA695 refers to the numbering system used in the Stibitz laboratory. The presence of pXO1 (but absence of cya) and the absence of pXO2 in NR-9398 has been confirmed by PCR amplification of plasmid-specific sequences from extracted DNA (see Table below).

Lot^{1,2}: 57969327

Manufacturing Date: 28NOV2008

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis Cellular morphology Colony morphology ³ Sporulation Motility β -hemolysis Capsule (India ink staining) ⁵ Tenacious Analytical profile index (API [®] 50 CHB/API [®] 20E) Nitrate reduction FAME analysis	Gram-positive rod Report results Positive Non-motile Non-hemolytic Negative Positive Consistent with <i>B. anthracis</i> Positive Consistent with <i>B. anthracis</i>	Gram-positive rod Flat, entire, ground-glass, gray (Figure 1) No spores observed ⁴ Non-motile Non-hemolytic Negative Positive Consistent with <i>B. anthracis</i> Positive Consistent with <i>B. anthracis</i>
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1420 base pairs)	Consistent with <i>Bacillus cereus</i> group	Consistent with <i>Bacillus cereus</i> group ⁶
PCR Assay of Extracted DNA 16S ribosomal RNA gene Specific chromosomal marker ⁷ Presence of virulence plasmids ⁸ pXO1 (three targets) pXO2 (three targets) Verification of Δ cya	~ 555 bp amplicon Amplicon present Amplicons present No amplicons No amplicon	~ 555 bp amplicon Amplicon present Amplicons present No amplicons No amplicon
Viability (post-vialing)³	Growth	Growth

¹*B. anthracis*, strain Sterne BA695 (Δ cya244) was deposited by E. Scott Stibitz, Division of Bacterial, Parasitic, and Allergenic Products, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland. NR-9398 was produced by inoculation of the deposited material into Tryptic Soy Broth and grown 24 hours at 37°C and 5% CO₂. Broth inoculum was added to Kolles which were grown 24 hours at 37°C and 5% CO₂ to produce this lot.

²A portion of this lot underwent brief thawing after being vialled. All Certificate of Analysis tests were performed on vials that had been thawed and those that were not. The results obtained from the thawed vials were identical to the results obtained from the vials that were not thawed.

³24 hours at 37°C and 5% CO₂ on Tryptic Soy Agar with 5% sheep blood

⁴Spores are not present unless exposed to low CO₂ levels, such as those found in the ambient atmosphere. Higher CO₂ levels inhibit spore formation.

⁵Virulent strains are positive for encapsulation.

⁶*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).

⁷This 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.

⁸Plasmids were verified using a proprietary (Patent Pending) PCR-based assay to a *Bacillus anthracis*-plasmids pXO1 and pXO2.

Figure 1



Date: 04 SEP 2012

Signature: 

Title: Technical Manager, BEI Authentication or designee

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