

Bacillus anthracis, Strain Sterne BA781 (Δ lef243/ Δ cya244/ Δ pagA242)

Catalog No. NR-9401

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Product Description: *Bacillus anthracis* (*B. anthracis*), strain Sterne BA781 (Δ lef243/ Δ cya244/ Δ pagA242) is a triple deletion mutant of the toxigenic acapsulate Sterne 7702 strain. The designation BA781 refers to the numbering system used in the Stibitz laboratory. The presence of pXO1 (but absence of the *lef*, *cya* and *pag* genes) and the absence of pXO2 in NR-9401 have been confirmed by PCR amplification of plasmid-specific sequences from extracted DNA (see Table below).

Lot¹: 57969330

Manufacturing Date: 30NOV2008

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis Cellular morphology Colony morphology ² Sporulation Motility β -hemolysis Capsule (India ink staining) ³ 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 Circular, low convex, ground-glass, grey (Figure 1) Positive 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 (~ 1410 base pairs)	Consistent with <i>B. cereus</i> group	Consistent with <i>B. cereus</i> group ⁴
PCR Assay of Extracted DNA 16S ribosomal RNA gene Specific chromosomal marker ⁵ Presence of virulence plasmids ⁶ pXO1 (one target) pXO2 (three targets) Verification of Δ lef/ Δ cya/ Δ pag	~ 555 bp amplicon Amplicon present Amplicon present No amplicons No amplicons	~ 555 bp amplicon Amplicon present Amplicon present No amplicons No amplicons
Viability (post-vialing)²	Growth	Growth

¹*B. anthracis*, strain BA781 (Δ lef243/ Δ cya244/ Δ pagA242) 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-9401 was produced by inoculation of the deposited material into Tryptic Soy Broth and grown 24 hours at 35°C and 5% CO₂. Broth inoculum was added to Kolles which were grown 24 hours at 35°C and 5% CO₂ to produce this lot.

²24 hours at 35°C and 5% CO₂ on Tryptic Soy Agar with 5% sheep blood

³Virulent strains are positive for encapsulation.

⁴*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).

⁵This 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.

⁶Plasmids 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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