

Certificate of Analysis for NR-10009

Bacillus anthracis, Strain Sterne ∆GBAA1941

Catalog No. NR-10009

Bacillus anthracis (B. anthracis), strain Sterne ΔGBAA1941 is a **Product Description:** markerless, nonpolar, 363 bp deletion mutant of the toxigenic acapsulate original Sterne strain (34F2). Nearly the entire open reading frame is replaced by three stop codons followed by two restriction endonuclease recognition sites. BamHI and Smal (to facilitate screening of the correct mutation). The first and last ten codons of the putative transcriptional regulator (GBAA1941) gene retain the wild type sequence.

Lot¹: 58441530 Manufacturing Date: 18DEC2008

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rod	Gram-positive rod
Colony morphology ²	Report results	Circular, flat, entire, ground-glass, grey, opaque (Figure 1)
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 including API [®] 20E; ONPG to GEL only)	Consistent with B. anthracis	Consistent with B. anthracis
Nitrate reduction	Positive	Positive
Genotypic Analysis ³ Sequencing of 16S ribosomal RNA gene (~ 1410 base pairs)	Consistent with Bacillus cereus group	Consistent with <i>Bacillus cereus</i> group ^{4,5}
PCR Assay of Extracted DNA ³ 16S ribosomal RNA gene Presence of virulence plasmids pXO1 (<i>aat</i>)	~ 1500 bp amplicon ~ 125 bp amplicon	~ 1500 bp amplicon ~ 125 bp amplicon
pXO1 (aai) pXO2 (at, capA, capB, capC)	No amplicons	No amplicons
μλοε (αι, ταμλ, ταμο, ταμο)	ino amplicons	140 amplicons
Viability (post-vialing) ⁶	Growth	Growth

¹B. anthracis, strain Sterne ∆GBAA1941 was deposited by Philip C. Hanna, Associate Professor, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan. NR-10009 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.

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²24 hours at 37°C on Tryptic Soy Agar with 5% sheep blood

³DNA was extracted from a broth culture produced from NR-10009, lot 58441530.

⁴Bacillus 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).

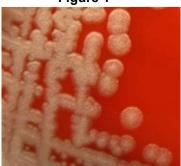
⁵Also consistent with *Bacillus coagulans* and *Bacillus subtilis*

⁶24 hours at 37°C in Tryptic Soy Broth



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Figure 1



Date: 19 NOV 2009 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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