

Certificate of Analysis for NR-9996

Bacillus anthracis, Strain Sterne AGBAA0552-2

Catalog No. NR-9996

Product Description: Bacillus anthracis (B. anthracis), strain Sterne \triangle GBAA0552-2 is a deletion mutant of the toxigenic acapsulate original Sterne strain (34F2), constructed by replacing codons 10 through 14 with three in-frame stop codons followed by the recognition site for BamHI (to facilitate screening of the correct mutation). The remainder of the putative internalin (GBAA0552-2) gene retains the wild type sequence.

Lot¹: 58394755 Manufacturing Date: 13NOV2008

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 Sequencing of 16S ribosomal RNA gene (~ 1420 base pairs)	Consistent with Bacillus cereus group	Consistent with Bacillus cereus group ^{3,4}
PCR Assay of Extracted DNA 16S ribosomal RNA gene Presence of virulence plasmids	~ 1500 bp amplicon	~ 1500 bp amplicon
pXO1 (aat)	~ 125 bp amplicon	~ 125 bp amplicon
pXO2 (at, capA, capB, capC)	No amplicons	No amplicons
Viability (post-vialing) ⁵	Growth	Growth

¹B. anthracis, strain Sterne ΔGBAA0552-2 was deposited by Philip C. Hanna, Associate Professor, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan. NR-9996 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.

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

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³Bacillus 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).

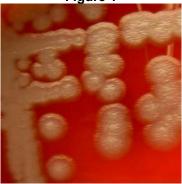
⁴Also consistent with Bacillus coagulans

⁵24 hours at 37°C in Tryptic Soy Broth



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Figure 1



Date: 05 MAY 2009 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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