

Certificate of Analysis for NR-10004

Bacillus anthracis, Strain Sterne △GBAA1975-76

Catalog No. NR-10004

Product Description: Bacillus anthracis (B. anthracis), strain Sterne ΔGBAA1975-76 is a markerless, nonpolar, double deletion mutant of the response regulator/histidine kinase genes from the toxigenic acapsulate original Sterne strain (34F2). The mutant retains the wild type sequence for the first six codons of GBAA1975, including the start codon, and the last six codons of GBAA1976. The restriction endonuclease recognition site for *Smal* was inserted between GBAA1975 and GBAA1976.

Lot¹: 58441525 Manufacturing Date: 18DEC2008

| TEST | SPECIFICATIONS | RESULTS |
|---|---------------------------------------|---|
| Phenotypic Analysis | | |
| Cellular morphology | Gram-positive rod | Gram-positive rod |
| Colony morphology ² | Report results | Circular, flat, 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 <i>B. anthracis</i> |
| Nitrate reduction | Positive | Positive |
| FAME analysis | Consistent with B. anthracis | Consistent with B. anthracis |
| Genotypic Analysis Sequencing of 16S ribosomal RNA gene | Consistent with Bacillus cereus group | Consistent with <i>Bacillus cereus</i> group ³ |
| (~ 1440 base pairs) | | |
| PCR Assay of Extracted DNA | | |
| 16S ribosomal RNA gene | ~ 1500 bp amplicon | ~ 1500 bp amplicon |
| Presence of virulence plasmids | | |
| pXO1 (aat) | ~ 125 bp amplicon | ~ 125 bp amplicon |
| pXO2 (at, capA, capB, capC) | No amplicons | No amplicons |
| Viability (post-vialing) ⁴ | Growth | Growth |

¹B. anthracis, strain Sterne ∆GBAA1975-76 was deposited by Philip C. Hanna, Associate Professor, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan. NR-10004 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

⁴24 hours at 37°C in Tryptic Soy Broth

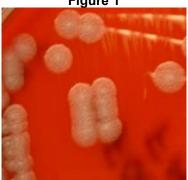
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³Bacillus cereus group species (*B. cereus*, *B. thuringiensis*, *B. mycoides*, and *B. anthracis*) cannot be classified based on 16S sequence (Spencer, R. C. "Bacillus anthracis." J. Clin. Pathol. 56 (2003): 182-187. PubMed: 12610093).



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Date: 24 AUG 2009 **Signature:** Signature on File

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