

Certificate of Analysis for NR-10000

Bacillus anthracis, Strain Sterne AGBAA0148-2

Catalog No. NR-10000

Product Description: Bacillus anthracis (B. anthracis), strain Sterne \triangle GBAA0148-2 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 spore germination gene (gerD; GBAA0148-2) retains the wild type sequence.

Lot¹: 58441521 Manufacturing Date: 17DEC2008

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) Nitrate reduction	Consistent with <i>B. anthracis</i> Positive	Consistent with <i>B. anthracis</i> Positive
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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	~ 1500 bp amplicon	~ 1500 bp amplicon
pXO1 (<i>aat</i>)	~ 125 bp amplicon	~ 125 bp amplicon
pXO2 (at, capA, capB, capC)	No amplicons	No amplicons
Viability (post-vialing) ⁶	Growth	Growth

¹B. anthracis, strain Sterne ∆GBAA0148-2 was deposited by Philip C. Hanna, Associate Professor, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan. NR-10000 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-10000, lot 58441521.

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*

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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