

Certificate of Analysis for NR-10008

Bacillus anthracis, Strain Sterne ∆GBAA1887-2

Catalog No. NR-10008

Product Description: Bacillus anthracis (B. anthracis), strain Sterne \triangle GBAA1887-2 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 enterotoxin gene (GBAA1887-2) retains the wild type sequence.

Lot¹: 58441529 Manufacturing Date: 07JAN2009

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rod	Gram-positive rod
Colony morphology ²	Report results	Circular, flat, entire, ground-glass, opaque and grey (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
FAME analysis	Consistent with <i>B. anthracis</i> and <i>B. cereus group species</i>	Consistent with <i>B. anthracis</i> and <i>B. cereus group species</i> ³
Genotypic Analysis ⁴		
Sequencing of 16S ribosomal RNA gene (~ 1390 base pairs)	Consistent with <i>B. anthracis</i> and <i>B. cereus group species</i>	Consistent with <i>B. anthracis</i> and <i>B. cereus group species</i> ^{5,6}
PCR Assay of Extracted DNA ⁴		
16S ribosomal RNA gene	~ 1500 bp amplicon	~ 1500 bp amplicon
Presence of virulence plasmids		·
pXO1 (<i>aat</i>)	~ 125 bp amplicon	~ 125 bp amplicon
pXO2 (at, capA, capB, capC)	No amplicons	No amplicons
Viability (post-vialing) ⁷	Growth	Growth

¹B. anthracis, strain Sterne ∆GBAA1887-2 was deposited by Philip C. Hanna, Associate Professor, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan. NR-10008 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

³FAME "Bacillus cereus group" includes the species B. anthracis, B. cereus, B. mycoides, B. pseudomycoides, B. thuringiensis and B. weihenstephanensis (Slabbinck, B., et al. "Genus-wide Bacillus Species Identification through Proper Artificial Neural Network Experiments on Fatty Acid Profiles." Antonie Van Leeuwenhoek 94 (2008): 187-198. PubMed: 18322819.)

⁴DNA was extracted from a broth culture produced from NR-10008 (Lot: 58441529).

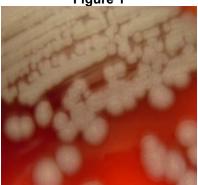
⁵Bacillus cereus group species (*B. cereus*, *B. thuringiensis*, *B. mycoides*, and *B. anthracis*) cannot be classified based on 16S sequence (Spencer, R. C. "Bacillus anthracis." J. Clin. Pathol. 56 (2003): 182-187. PubMed: 12610093).

⁶Also consistent with *B. subtilis*

⁷24 hours at 37°C in Tryptic Soy Broth

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