

Certificate of Analysis for NR-10005

Bacillus anthracis, Strain Sterne △GBAA0650-51

Catalog No. NR-10005

Product Description: Bacillus anthracis (B. anthracis), strain Sterne \triangle GBAA0650-51 is a markerless, nonpolar, double deletion mutant of the response regulator/histidine kinase genes from the toxigenic acapsulate original Sterne strain (34F2). This mutant retains the first 100 codons of the histidine kinase gene (GBAA0651) followed by two stop codons and the restriction endonuclease recognition site for *Smal* followed by the last 100 codons of the response regulator gene (GBAA0650).

Lot¹: 58441526 Manufacturing Date: 19DEC2008

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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) Nitrate reduction FAME analysis	Consistent with <i>B. anthracis</i> Positive	Consistent with <i>B. anthracis</i> Positive
Bioterrorism bacteria library (BTR3 3.00)	Consistent with B. anthracis	Consistent with B. anthracis
Clinical bacteria library (CLIN6 6.00)	Consistent with B. cereus group	Consistent with <i>B. cereus</i> group ³
Genotypic Analysis ⁴		
Sequencing of 16S ribosomal RNA gene (~ 1350 base pairs)	Consistent with <i>B. anthracis</i> and <i>B. cereus</i> group	Consistent with <i>B. anthracis</i> and <i>B. cereus</i> group ⁵
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) ⁶	Growth	Growth

B. anthracis, strain Sterne ΔGBAA0650-51 was deposited by Philip C. Hanna, Associate Professor, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan. NR-10005 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.

⁶24 hours at 37°C in Tryptic Soy Broth

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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).

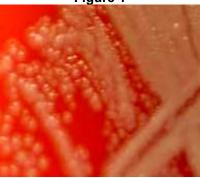
⁴DNA was extracted from a broth culture produced from NR-10005 (Lot: 58441526).

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).



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Date: 28 AUG 2009 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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