

***Bacillus anthracis*, Strain Sterne ΔGBAA0552-2, ΔGBAA1346-2**

Catalog No. NR-9998

Product Description: *Bacillus anthracis* (*B. anthracis*), strain Sterne ΔGBAA0552-2, ΔGBAA1346-2 is a double 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 *Bam*HI (to facilitate screening of the correct mutation). The remainder of the putative internalin genes (GBAA0552-2 and GBAA1346-2) retains the wild type sequence.

Lot¹: 58394757

Manufacturing Date: 05NOV2008

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis Cellular morphology Colony morphology Tryptic Soy Agar, 5% sheep blood ² PLET Agar ^{2,3} Sporulation Motility β-hemolysis Capsule (India ink staining) Tenacious Analytical profile index (API [®] 50 CHB including API [®] 20E; ONPG to GEL only) Nitrate reduction	Gram-positive rod Report results Report results Positive Non-motile Non-hemolytic Negative Positive Consistent with <i>B. anthracis</i> Positive	Gram-positive rod Circular, low convex, erose, ground-glass, opaque and grey (Figure 1) Circular, flat, lobate, ground-glass, opaque and cream (Figure 2) Positive Non-motile Non-hemolytic Negative Positive Consistent with <i>B. anthracis</i> Positive
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1460 base pairs)	Consistent with <i>Bacillus cereus</i> group	Consistent with <i>Bacillus cereus</i> group ⁴
PCR Assay of Extracted DNA 16S ribosomal RNA gene Presence of virulence plasmids pXO1 (<i>aat</i>) pXO2 (<i>at</i> , <i>capA</i> , <i>capB</i> , <i>capC</i>)	~ 1500 bp amplicon ~ 125 bp amplicon No amplicons	~ 1500 bp amplicon ~ 125 bp amplicon No amplicons
Viability (post-vialing)⁵	Growth	Growth

¹*B. anthracis*, strain Sterne ΔGBAA0552-2, ΔGBAA1346-2 was deposited by Philip C. Hanna, Associate Professor, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan. NR-9998 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

³Growth on PLET [polymyxin-lysozyme-EDTA-thallos acetate] Agar (Hardy Diagnostics, Cat. No. G153) differentiates *B. anthracis* from other *Bacillus* species, including *B. cereus*, *B. thuringiensis* and *B. mycooides*, whose growth is inhibited by the combination of EDTA and thallium cations. Dragon, D. C. and R. P. Rennie. "Evaluation of Spore Extraction and Purification Methods for Selective Recovery of Viable *Bacillus anthracis* Spores." *Lett. Appl. Microbiol.* 33 (2001): 100-105. PubMed: 11472515.

⁴*Bacillus cereus* group species (*B. cereus*, *B. thuringiensis*, *B. mycooides*, and *B. anthracis*) cannot be classified based on 16S sequence (Spencer, R. C. "*Bacillus anthracis*." *J. Clin. Pathol.* 56 (2003): 182-187. PubMed: 12610093).

⁵24 hours at 37°C in Tryptic Soy Broth

Figure 1

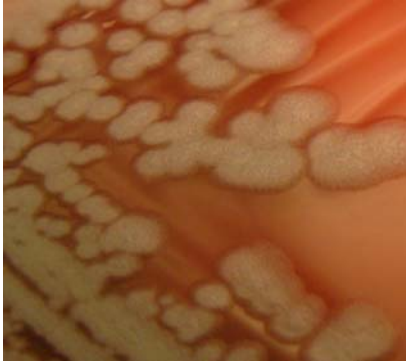
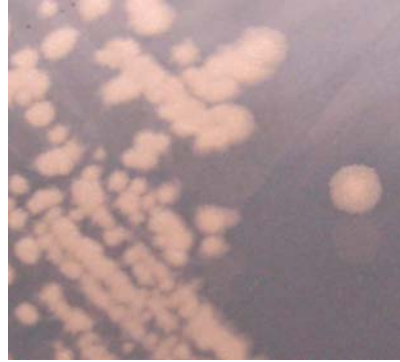


Figure 2



Date: 23 JUN 2009

Signature: Signature on File

Title: Technical Manager, BEI Authentication or designee

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