b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-9992

Bacillus anthracis, Strain Sterne 34F2, BA854 (Δ*asbD*)

Catalog No. NR-9992

Product Description: *Bacillus anthracis* (*B. anthracis*), strain Sterne 34F2, BA854 (Δ*asbD*) was deposited as a markerless, nonpolar, 231 base pair deletion mutant of *asbD*, a member of the petrobactin biosynthetic operon (*asbABCDEF*).

Lot[1]: 58394748 **Manufacturing Date: 05NOV2008**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology[2]	Report results	Circular, low convex, entire, ground-glass, opaque and gray (Figure 1)
Sporulation	Report results	No spores observed
Hemolysis[2]	Non-hemolytic	Non-hemolytic
Motility	Non-motile	Non-motile
Capsule (India ink staining)	Negative	Negative
Tenacious	Positive	Positive
Analytical profile index (API® 50 CHB including API® 20E; ONPG to GEL only)	*B. anthracis* (≥ 80%)	*B. anthracis* (38.6%)[3]
Nitrate reduction	Positive	Positive
Genotypic Analysis		
Sequencing of 16S ribosomal RNA (rRNA) gene (~ 1420 base pairs)	> 99% identical to *B. anthracis*, strain Sterne (GenBank: AE017225)	99.9% identical to *B. anthracis*, strain Sterne (GenBank: AE017225)[4]
PCR Amplification of *B. anthracis* specific chromosomal region[5]	~ 200 base pair amplicon	~ 200 base pair amplicon
***Bacillus anthracis* specific prophage PCR[6]**		
16S rRNA gene	Amplicon present	Amplicon present
Prophage 1	Report results	Amplicon present
Prophage 2	Report results	Amplicon present
Prophage 3	Report results	Amplicon present
Prophage 4	Report results	Amplicon present
Presence of Plasmids Confirmed by PCR Amplification[7,8]		
16S rRNA gene	Amplicon present	Amplicon present
pXO1 (four targets)	Amplicons present	Amplicons present
pXO2 (three targets)	No amplicons	No amplicons
Viability (post-freeze)[2]	Growth	Growth

[1]NR-9992 was produced by inoculation of the deposited material into Brain Heart Infusion broth and grown 1 day at 30°C in an aerobic atmosphere. 10% glycerol was added to the resulting growth and the mixture was cryopreserved. A vial of the preserved material was thawed and used to inoculate a tube of Tryptic Soy broth and grown 1 day at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar with 5% defibrinated sheep blood kolles which were grown 1 day at 37°C in an aerobic atmosphere to produce this lot.

[2]1 day at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood

[3]99.8% probability that the genus is *Bacillus*; 38.6% *B. anthracis*, 34% *B. mycoides* and 27.2% *B. cereus*.

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

[5]This product was verified to a species level using a PCR-based assay to a *B. anthracis*-specific genetic mutation capable of differentiating *B. anthracis* from the remainder of the *B. cereus* group.

[6]The prophage-specific multiplex PCR detects 4 prophages that only *B. anthracis* contains, no other species of *Bacillus* has been known to contain more than one prophage (Sozhamannan, S., et al. "The *Bacillus anthracis* Chromosome Contains Four Conserved, Excision-Proficient, Putative Prophages." BMC Microbiol. 6 (2006): 34. PubMed: 16600039).

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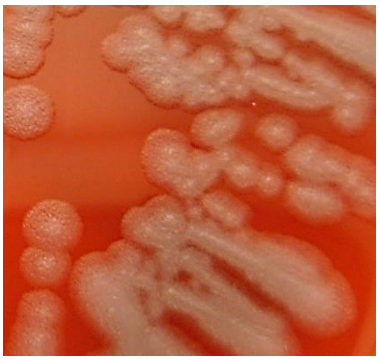
[7]For PCR primers used in these assays, refer to Riojas, M. A., et al. "Multiplex PCR for Species-Level Identification of *Bacillus anthracis* and Detection of pXO1, pXO2, and Related Plasmids." Health Security 13 (2015): 122-129. PubMed: 25813976.
[8]Plasmids were verified using a PCR-based assay to *B. anthracis*-plasmids pXO1 and pXO2.

Figure 1: Colony Morphology



Date: 18 JAN 2017 **Signature:**

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