

Certificate of Analysis for NR-13671

Bacillus anthracis, Strain Sterne 34F₂, Derivative KDC7 (∆bacACEBF)

Catalog No. NR-13671

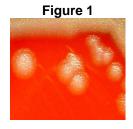
Product Description: Bacillus anthracis (B. anthracis), strain Sterne 34F₂, derivative KDC7, is a deletion mutant of the bacillibactin biosynthetic pathway genes ($\triangle bacACEBF$).

Lot¹: 62849232 Manufacturing Date: 14AUG2014

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ²	Report results	Circular, low convex, undulate, rough and gray (Figure 1)
Hemolysis ³	Non-hemolytic	Non-hemolytic
Motility ⁴	Non-motile Non-motile	Non-motile
Biochemical characterization:		
Nitrate reduction	Positive	Positive
Arginine decarboxylase	Report results	Negative
Production of acid from trehalose	Positive	Positive
Production of acid from salicin	Negative	Negative
Production of acid from glycerol	Negative	Negative
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1500 base pairs)	Consistent with Bacillus cereus group	Consistent with Bacillus cereus group ⁵
PCR Assay of Extracted DNA		
16S ribosomal RNA gene	~ 560 bp amplicon	~ 560 bp amplicon
Species specific chromosomal marker ⁶	Amplicon present	Amplicon present
Presence of virulence plasmids ⁷		
pXO1 (four targets)	Amplicons present	Amplicons present
pXO2 (three targets)	No amplicons	No amplicons
Purity (post-freeze) ⁸	Growth consistent with B. anthracis	Growth consistent with B. anthracis
Viability (post-freeze) ²	Growth	Growth

¹NR-13671 was produced by inoculation of the deposited material into Tryptic Soy broth and grown 24 hours at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar with 5% defibrinated sheep blood kolles which were grown 22 hours at 37°C in an aerobic atmosphere to produce this lot.

⁸Purity of this lot was assessed for 7 days under propagation conditions on Tryptic Soy agar with 5% defibrinated sheep blood.



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²22 hours at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood

³24 hours at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood

⁴48 hours at 37°C in an aerobic atmosphere on motility test media with triphenyltetrazolium chloride (TTC). In the *B. cereus* group, *B. cereus* and *B. thuringiensis* are motile, whereas *B. anthracis* and *B. mycoides* are non-motile.

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).

⁶This product was verified to a species level using a proprietary (Patent Pending) PCR-based assay to *Bacillus anthracis*-specific genetic mutation capable of differentiating *B. anthracis* from the remainder of the *B. cereus* group.

Plasmids were verified using a proprietary (Patent Pending) PCR-based assay to Bacillus anthracis-plasmids pXO1 and pXO2.



Certificate of Analysis for NR-13671

Date: 10 DEC 2014

Signature: (

Title:

Technical Manager, BEI Authentication or designee

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