SUPPORTING INFECTIOUS DISEASE RESEARCH

Bacillus anthracis, Strain Sterne 7702, Derivative BDT101

Catalog No. NR-13673

Product Description: Bacillus anthracis (B. anthracis), strain Sterne 7702, derivative BDT101, was deposited as an anthrolysin O (ALO) deletion mutant where the *aloA* gene was replaced with a kanamycin resistance (Km^r) cassette.

Lot¹: 63831078

Manufacturing Date: 19NOV2015

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ²	Report results	Irregular, low convex, undulate, ground-glass and gray (Figure 1)
Motility ³	Non-motile	Non-motile
Hemolysis ²	Non-hemolytic	Non-hemolytic
Tenacious	Positive	Positive
Biochemical characterization:		
Nitrate reduction	Positive	Positive
Arginine dihydrolase	Negative	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 (rRNA) gene (~ 1370 base pairs)	> 99% identical to <i>B. anthracis</i> , strain Sterne (GenBank: AE017225)	100% identical to <i>B. anthracis</i> , strain Sterne (GenBank: AE017225) ⁴
PCR Amplification of <i>B. anthracis</i> specific chromosomal region ⁵	~ 200 base pair amplicon	~ 200 base pair amplicon
Presence of Plasmids Confirmed by PCR Amplification ^{6,7}		
16S rRNA gene	Amplicon present	Amplicon present
pXO1 (four targets)	Amplicons present	Amplicons present
pXO2 (three targets)	No amplicons	No amplicons
Confirmation of Kanamycin Resistance ⁸	Growth	Growth
Purity (post-freeze) ⁹	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze) ²	Growth	Growth

¹NR-13673 was produced by inoculation of the deposited material into Tryptic Soy broth and grown 1 day at 37°C in an aerobic atmosphere with 5% CO₂. 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 with 5% CO₂ to produce this lot.

²1 day at 37°C in an aerobic atmosphere with 5% CO₂ on Tryptic Soy agar with 5% defibrinated sheep blood

³Motility test was performed on TTC media (Remel RO61414) for 7 days at 37°C in an aerobic atmosphere. In the *B. cereus* group, *B. cereus* and *B. thuringiensis* are motile, whereas *B. anthracis* and *B. mycoides* are non-motile.

⁴Also 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*." <u>J. Clin. Pathol.</u> 56 (2003): 182-187. PubMed: 12610093).

⁵This 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.

⁶For 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." <u>Health Security</u> 13 (2015): 122-129. PubMed: 25813976.

⁷Plasmids were verified using a PCR-based assay to *B. anthracis*-plasmids pXO1 and pXO2.

⁸1 day at 37°C in an aerobic atmosphere on Luria-Bertani agar with 50 µg/mL kanamycin

⁹Purity of this lot was assessed for 14 days at 37°C in an aerobic atmosphere with 5% CO₂ on Tryptic Soy agar with 5% defibrinated sheep blood.

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Certificate of Analysis for NR-13673

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Figure 1: Colony Morphology

Date: 19 JAN 2017

Signature: (

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