

**Bacillus anthracis, Strain Sterne 7702, Derivative BJH035**

**Catalog No. NR-13674**

**Product Description:** *Bacillus anthracis* (*B. anthracis*), strain Sterne 7702, derivative BJH035, was deposited as a deletion mutant of a lethal factor precursor gene where *lef* was replaced with a kanamycin resistance (Km<sup>r</sup>) cassette.

**Lot<sup>1</sup>: 64184815**

**Manufacturing Date: 15APR2016**

TEST	SPECIFICATIONS	RESULTS
<b>Phenotypic Analysis</b> Cellular morphology Colony morphology <sup>2</sup>  Hemolysis <sup>2</sup> Motility <sup>3</sup> Biochemical characterization: Nitrate reduction Arginine dihydrolase Production of acid from trehalose Production of acid from salicin Production of acid from glycerol	Gram-positive rods Report results  Non-hemolytic Non-motile  Positive Report results Positive Negative Negative	Gram-positive rods Irregular, flat, undulate, ground-glass and gray (Figure 1) Non-hemolytic Non-motile  Positive Negative Positive Negative Negative
<b>Genotypic Analysis</b> Sequencing of 16S ribosomal RNA gene (~ 1490 base pairs)	> 99% identical to <i>B. anthracis</i> , strain Sterne (GenBank: AE017225)	99.9% identical to <i>B. anthracis</i> , strain Sterne (GenBank: AE017225) <sup>4</sup>
<b>PCR Assay of Extracted DNA</b> 16S ribosomal RNA gene	~ 560 base pair amplicon	~ 560 base pair amplicon
<b>PCR Amplification of <i>B. anthracis</i> specific chromosomal region<sup>5</sup></b>	~ 200 base pair amplicon	~ 200 base pair amplicon
<b>Presence of Plasmids Confirmed by PCR Amplification<sup>6,7</sup></b> pXO1 (four targets) pXO2 (three targets)	Amplicons present No amplicons	Amplicons present <sup>8</sup> No amplicons
<b>Presence of Kanamycin Resistance<sup>9</sup></b>	Confirmed	Confirmed
<b>Purity (post-freeze)<sup>10</sup></b>	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
<b>Viability (post-freeze)<sup>2</sup></b>	Growth	Growth

<sup>1</sup>NR-13674 was produced by inoculation of the deposited material into 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.

<sup>2</sup>1 day at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood

<sup>3</sup>Motility test was performed on TTC media (Remel RO61414) for 14 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.

<sup>4</sup>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).

<sup>5</sup>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.

<sup>6</sup>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.

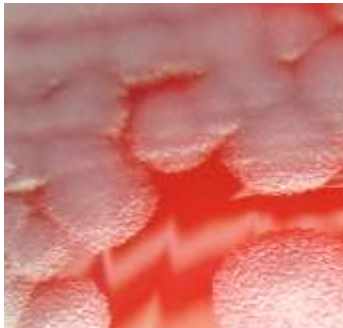
<sup>7</sup>Plasmids were verified using a PCR-based assay to *B. anthracis*-plasmids pXO1 and pXO2.

<sup>8</sup>Although this item was deposited as having a *lef* deletion, our in house PCR indicates *lef* is present (Heffernan, B. J., et al. "Bacillus anthracis Phospholipases C Facilitate Macrophage-Associated Growth and Contribute to Virulence in a Murine Model of Inhalation Anthrax." *Infect. Immun.* 74 (2006): 3756-3764. PubMed: 16790747.) The item has been shown to be kanamycin resistant, despite the presence of *lef*.

<sup>9</sup>1 day at 37°C in an aerobic atmosphere on Luria-Bertani agar with 50 µg/mL kanamycin

<sup>10</sup>Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.

Figure 1: Colony Morphology



Date: 20 JAN 2017

Signature:

A handwritten signature in black ink, appearing to read "David C. Cook". The signature is written in a cursive, flowing style.

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