

Certificate of Analysis for NR-1151

Bacillus anthracis, Strain V770-NP1-R (A0267)

Catalog No. NR-1151

Product Description: Bacillus anthracis (B. anthracis), strain V770-NP1-R (A0267) is a laboratory-derived non-proteolytic, non-encapsulated mutant of the Vollum strain. It was obtained by the Lawrence Livermore National Laboratory from USAMRIID.

Lot¹: 60951327 Manufacturing Date: 30MAY2012

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rod	Gram-positive rod
Colony morphology ²	Report results	Irregular, flat, undulate, opaque and white (Figure 1)
Motility ³	Non-motile	Non-motile
β-hemolysis	Non-hemolytic	Non-hemolytic
Tenacious	Positive	Positive
Biochemical characterization		
Production of acid from trehalose	Positive	Positive
Production of acid from salicin	Negative	Negative
Production of acid from glycerol	Negative	Negative
Nitrate reduction	Positive	Positive
Arginine dihydrolase	Negative	Negative
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (1400 base pairs)	Consistent with <i>Bacillus cereus</i> group ⁴	Consistent with Bacillus cereus group ⁴
PCR Assay of Extracted DNA		
16S ribosomal RNA gene	~ 555 base pair amplicon	~ 555 base pair amplicon
B. anthracis specific chromosomal marker ^{5,6}	Amplicon present	Amplicon present
Presence of virulence plasmids ^{6,7}		·
pXO1 (four targets)	Amplicons present	Amplicons present
pXO2 (three targets)	No amplicons present	No amplicons present
Purity (post-freeze) ⁸	Growth consistent with B. anthracis	Growth consistent with B. anthracis
Viability (Post-vialing) ²	Growth	Growth

B. anthracis, strain V770-NP1-R (A0267) was produced by inoculation of the deposited material into Tryptic Soy broth and grown 24 hours at 35°C in an aerobic atmosphere with 5% CO₂ and the material was frozen after the addition of glycerol. The frozen material from the initial growth was then used to inoculate Tryptic Soy broth and grown for 5 days at 37°C in an aerobic atmosphere with 5% CO₂. Broth inoculum from the second passage was added to kolles which were grown 24 hours at 37°C in an aerobic atmosphere with 5% CO₂ to produce this lot.

BEI Resources www.beiresources.org E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898

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

³Motility test performed on Remel™ Motility Test Medium w/TTC indicator for 7 days at 37°C in an aerobic atmosphere with 5% CO₂. 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." Health Security 13 (2015): 122-129. PubMed: 25813976.

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

⁸Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere with 5% CO₂ on Tryptic Soy agar with 5% defibrinated sheep blood.



Certificate of Analysis for NR-1151

Figure 1: Colony Morphology



Date: 11 SEP 2015

Signature: Jantina M. Hetygan

Title: Senior Director, Compliance & QA/Chief Compliance Officer

ATCC[®], on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC[®]'s knowledge.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

NR-1151 60951327 11SEP2015