

Certificate of Analysis for NR-13594

Genomic DNA from Clostridium difficile, Isolate 3

Catalog No. NR-13594

Product Description: Genomic DNA was obtained from a preparation of *Clostridium difficile* (*C. difficile*), isolate 3, which was isolated from a human patient from the Mid-Atlantic region of the United States in 2008/2009.

Lot¹: 58791985 Manufacturing Date: 21OCT2010

TEST	SPECIFICATIONS	RESULTS
Sequencing of 16S Ribosomal RNA Gene (~ 1220 base pairs)	Consistent with C. difficile	Consistent with C. difficile
Agarose Gel Electrophoresis	High molecular weight chromosomal DNA	High molecular weight chromosomal DNA (Figure 1)
Concentration by PicoGreen® Measurement	0.7 to 1.5 μg in 25 to 100 μL per vial	1.2 μg in 69 μL per vial (17 μg/mL)
Functional Activity by PCR Amplification 16S ribosomal RNA gene Presence of <i>C. difficile</i> -specific genes ² Triose phosphate isomerase (<i>tpi</i>) Toxin A (<i>tcdA</i>)	~ 1500 bp amplicon ~ 230 bp amplicon ~ 369 bp amplicon	~ 1500 bp amplicon ~ 230 bp amplicon ~ 369 bp amplicon
OD ₂₆₀ /OD ₂₈₀ Ratio	1.7 to 2.0	1.7
Bacterial Inactivation 10% of total yield plated on CDC anaerobic blood agar ^{3,4}	No viable bacteria detected	No viable bacteria detected

¹The bacterial preparation used for extraction of genomic DNA was produced by Modified Reinforced Clostridial broth culture of the third passage of NR-13429 lot 58771085. After incubation for 48 hours at 37°C in an anaerobic atmosphere (80% N₂:10% CO₂:10% H₂), genomic DNA was extracted using proprietary technology. The deposited material was initially grown in Modified Reinforced Clostridial broth and incubated for 48 hours at 37°C and anaerobic atmosphere (80% N₂:10% CO₂:10% H₂) and was passaged three times under equivalent conditions.

Date: 09 JAN 2014 **Signature:**

Title: Technical Manager, BEI Authentication or designee

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.

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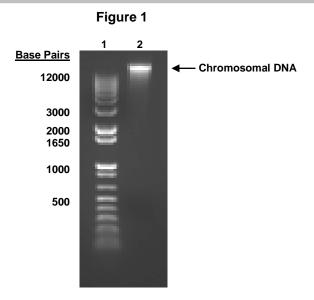
²Lemee, L., et al. "Multiplex PCR Targeting *tpi* (Triose Phosphate Isomerase), *tcdA* (Toxin A), and *tcdB* (Toxin B) Genes for Toxigenic Culture of Clostridium difficile." J. Clin. Microbiol. 42 (2004): 5710-5714. PubMed: 15583303.

³7 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% sheep blood.

⁴An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-negative and Gram-positive bacteria.



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Lane 1: Invitrogen™ TrackIt 1 Kb Plus DNA Ladder™

Lane 2: 200 ng of NR-13594

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