

***Clostridium difficile*, Isolate 20100432**

Catalog No. NR-49298

Product Description: *Clostridium difficile* (*C. difficile*; also referred to as *Peptoclostridium difficile*), isolate 20100432 was obtained from the stool of a female patient with a healthcare-associated (HA) *C. difficile* infection in midwestern USA in 2010. Isolate 20100432 was deposited as PCR ribotype 020, North American pulsed-field gel electrophoresis type 4 (NAP4), containing *tcdA*, *tcdB* and *tcdC* of the PaLoc operon. This isolate is reported to be negative for the *C. difficile* binary toxin (CDT).

Lot¹: 63719824

Manufacturing Date: 24SEP2015

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis Cellular morphology Colony morphology ² Motility (wet mount) Biochemical tests: Esculin hydrolysis ³ Gelatin hydrolysis ³ VITEK [®] MS (MALDI-TOF)	Gram-positive rods Report results Report results Positive Positive Consistent with <i>C. difficile</i>	Gram-positive rods Irregular, flat, lobate and gray (Figure 1) Motile Positive Positive <i>C. difficile</i> (99.9%)
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1440 base pairs)	≥ 99% sequence identity to <i>C. difficile</i> type strain (GenBank: CP011968.1)	99.9% sequence identity to <i>C. difficile</i> type strain (GenBank: CP011968.1)
PCR Assay of Extracted DNA Presence of <i>C. difficile</i> -specific genes ⁴ Triose phosphate isomerase (<i>tpi</i>) Presence of toxin genes ^{4,5} <i>cdtB</i> <i>tcdA</i> (wild type) <i>tcdA</i> (partial deletion) <i>tcdB</i>	~ 230 base pair amplicon No amplicon ~ 370 base pair amplicon No amplicon ~ 160 base pair amplicon	~ 230 base pair amplicon No amplicon ~ 370 base pair amplicon No amplicon ~ 160 base pair amplicon
Purity (post-freeze) Anaerobic growth ⁶ Aerobic growth ⁷	Consistent with expected colony morphology No growth	Consistent with expected colony morphology No growth
Viability (post-freeze)²	Growth	Growth

¹NR-49298 was produced by inoculation of the deposited material into Modified Reinforced Clostridial medium and incubated for 1 day at 37°C in an anaerobic atmosphere (< 0.5% O₂; Remel™ Anaero Pack-Anaero™ R681001). The material from the initial growth was passaged once in Modified Reinforced Clostridial medium for 1 day at 37°C in an anaerobic atmosphere to produce this lot.

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

³Tests were assessed after 7 days at 37°C in an anaerobic atmosphere. The gelatin tube was placed at 4°C for one hour prior to result determination.

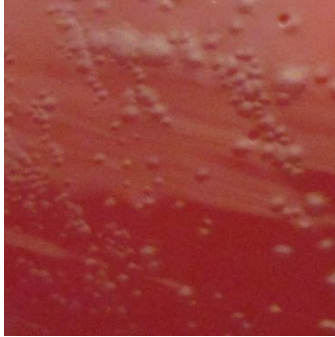
⁴Lemee, L., et al. "Multiplex PCR Targeting *tpi* (Triose Phosphate Isomerase), *tcdA* (Toxin A), and *tcdB* (Toxin B) Genes for Toxigenic Culture of *Clostridium difficile*." *J. Clin. Microbiol.* 42 (2004): 5710-5714. PubMed: 15583303.

⁵Antikainen, J., et al. "Detection of Virulence Genes of *Clostridium difficile* by Multiplex PCR." *APMIS*. 117 (2009): 607-613. PubMed: 19664132.

⁶Purity of this lot was assessed for 2 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.

⁷Purity of this lot was assessed for 9 days at 37°C in an aerobic atmosphere with 5% CO₂ on Tryptic Soy agar with 5% defibrinated sheep blood.

Figure 1: Colony Morphology



Date: 14 OCT 2016

Signature:

A handwritten signature in black ink, appearing to read "David C. Cook".

BEI Resources Authentication

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.

