

***Clostridioides difficile*, Isolate 20100584**
Catalog No. NR-49294
Product Description:

Clostridioides difficile (*C. difficile*), isolate 20100584 was obtained from the stool of an elderly adult male patient with a healthcare-associated (HA) *C. difficile* infection in western USA, in 2010. Previously referred to as *Clostridium difficile*, this genus has been reclassified and the genus designation on the vial label refers to the old nomenclature. Isolate 20100584 was deposited as PCR ribotype 014, 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). NR-49294 was produced by inoculation of BEI Resources seed lot 63719812 into Modified Reinforced Clostridial broth and grown for 3 days at 37°C in an anaerobic atmosphere (< 5% O₂; Remel™ Pack-Anaero™). Growth inoculum was added to Tryptic Soy Agar with 5% defibrinated sheep blood kolles, which were grown for 2 days at 37°C in an anaerobic atmosphere to produce this lot. Quality control testing was completed under propagation conditions unless otherwise noted.

Lot: 70066866
Manufacturing Date: 06MAR2024

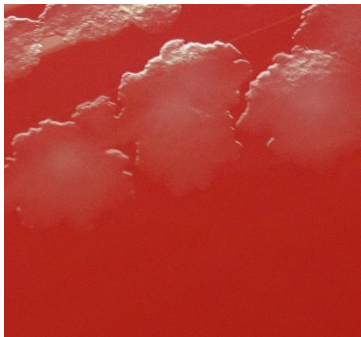
TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis Cellular morphology Colony morphology 3 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood Motility (wet mount) Hemolysis 3 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood VITEK® MS (MALDI-TOF) Biochemical Characterization Esculin hydrolysis ¹ Gelatin hydrolysis ¹	Gram-positive rods Report results Motile Report results <i>C. difficile</i> Positive Positive	Gram-positive rods Irregular, flat, undulate, opaque and gray (Figure 1) Motile Non-hemolytic <i>C. difficile</i> (99.9%) Positive Positive
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1440 base pairs)	≥ 99% sequence identity to <i>C. difficile</i> type strain (GenBank: MUJVV01000001)	100% sequence identity to <i>C. difficile</i> type strain (GenBank: MUJVV01000001)
PCR Assay of Extracted DNA Presence of <i>C. difficile</i> -specific genes ² Triose phosphate isomerase (<i>tpi</i>) Presence of toxin genes ^{2,3} <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 7 days at 37°C on Tryptic Soy agar with 5% defibrinated sheep blood Aerobic with 5% CO ₂ 7 days at 37°C on Tryptic Soy agar with 5% defibrinated sheep blood	Growth consistent with expected colony morphology No growth	Growth consistent with expected colony morphology No growth
Viability (post-freeze) 3 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood	Growth	Growth

¹Tests were assessed after 7 days at 37°C in an anaerobic atmosphere.

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.

³Antikainen, J., et al. "Detection of Virulence Genes of *Clostridium difficile* by Multiplex PCR." *APMIS* 117 (2009): 607-613. PubMed: 19664132.

Figure 1: Colony Morphology



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