

Certificate of Analysis for NR-43537

Peptoclostridium difficile, Strain CD18

Catalog No. NR-43537

Product Description: Peptoclostridium difficile (P. difficile; also referred to as Clostridium difficile), strain CD18 is a toxigenic strain isolated in January 2010 from the stool of a patient with an acute Clostridium difficile infection (CDI) in Ann Arbor, Michigan, USA.

Lot1: 64463690 Manufacturing Date: 11AUG2016

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ²	Report results	Irregular, flat, undulate, opaque
		and gray (Figure 1)
Hemolysis ²	Report results	Non-hemolytic
Motility (wet mount)	Report results	Motile
Biochemical tests		
Esculin hydrolysis	Positive	Positive
Gelatin hydrolysis	Positive	Positive
VITEK® MS (MALDI-TOF)	P. difficile	P. difficile (99.9%)
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene	≥ 99% sequence identity to	99.7% sequence identity to
(~ 370 base pairs)	P. difficile, strain CD18	P. difficile, strain CD18
, ,	(GenBank: AVGN01000041.1)	(GenBank: AVGN01000041.1)
(~ 810 base pairs)	Consistent with P. difficile	Consistent with P. difficile
PCR Assay of Extracted DNA		
Presence of <i>P. difficile</i> -specific genes ³		
Triose phosphate isomerase (tpi)	~ 230 base pair amplicon	~ 230 base pair amplicon
Presence of toxin genes ^{3,4}		
cdtB	~ 510 base pair amplicon	~ 510 base pair amplicon
tcdA (wild type)	No amplicon	No amplicon
tcdA (partial deletion)	No amplicon	No amplicon
tcdB	~ 160 base pair amplicon	~ 160 base pair amplicon
Purity (post-freeze)		
Anaerobic growth ⁵	Growth consistent with expected	Growth consistent with expected
	colony morphology	colony morphology
Aerobic growth ⁶	No growth	No growth
Viability (post-freeze) ²	Growth	Growth

¹NR-43537 was produced by inoculation of the deposited material into Modified Reinforced Clostridial medium and incubated for 2 days 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.

Page 1 of 2

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

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

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

³Lemee, 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

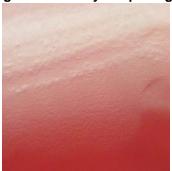
⁴Antikainen, J., et al. "Detection of Virulence Genes of Clostridium difficile by Multiplex PCR." APMIS. 117 (2009): 607-613. PubMed: 19664132 ⁵Purity of this lot was assessed for 7 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.

⁶Purity 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-43537

Figure 1: Colony Morphology



Date: 15 DEC 2016

Signature:

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.

 $\mbox{ATCC}^{\mbox{\tiny{\$}}}$ 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.

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

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