

## Certificate of Analysis for NR-43529

## Peptoclostridium difficile, Strain CD133

## Catalog No. NR-43529

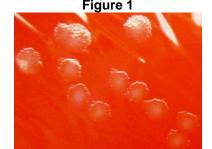
**Product Description:** Peptoclostridium difficile (P. difficile; also referred to as Clostridium difficile), strain CD133 is a toxigenic strain isolated in March 2010 from the stool of a human patient diagnosed with an acute Clostridium difficile infection in Ann Arbor, Michigan, USA.

Lot<sup>1</sup>: 63261378 Manufacturing Date: 15JAN2015

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology <sup>2</sup>	Report results	Irregular, slight peaked undulate, smooth and gray (Figure 1)
Hemolysis <sup>2</sup>	Report results	Non-hemolytic
Motility (wet mount)	Report results	Motile
Biochemical tests:		
Esculin hydrolysis	Positive	Positive
Gelatin hydrolysis	Positive	Positive
VITEK® MS (MALDI-TOF)	Consistent with P. difficile	Consistent with P. difficile
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1450 base pairs)	Consistent with P. difficile	Consistent with <i>P. difficile</i> <sup>3</sup>
PCR Assay of Extracted DNA  Presence of <i>P. difficile</i> -specific genes <sup>4</sup> Triose phosphate isomerase ( <i>tpi</i> )  Presence of toxin genes <sup>4</sup>	~ 230 base pairs amplicon	~ 230 base pairs amplicon
Toxin A (tcdA)	~ 370 base pairs amplicon	~ 370 base pairs amplicon
Toxin B ( <i>tcdB</i> )	~ 160 base pairs amplicon	~ 160 base pairs amplicon
Purity (post-freeze) Anaerobic growth <sup>5</sup> Aerobic growth <sup>6</sup>	Growth consistent with <i>P. difficile</i> No growth	Growth consistent with <i>P. difficile</i> No growth
Viability (post-freeze) <sup>2</sup>	Growth	Growth

NR-43529 was produced by inoculation of the deposited material into Modified Reinforced Clostridial medium and incubated for 24 hours 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 24 hours under propagation conditions to produce this lot.

<sup>&</sup>lt;sup>6</sup>Purity of this lot was assessed for 7 days on Tryptic Soy agar with 5% defibrinated sheep blood in an aerobic atmosphere with 5% CO<sub>2</sub>.



BEI Resources

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<sup>&</sup>lt;sup>2</sup>23 hours on Tryptic Soy agar with 5% defibrinated sheep blood under propagation conditions

<sup>&</sup>lt;sup>3</sup>≥ 99.7% identical to GenBank: AVHS01000113.1 (*P. difficile*, strain CD133)

<sup>&</sup>lt;sup>4</sup>Lemee, 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.

<sup>&</sup>lt;sup>5</sup>Purity of this lot was assessed for 7 days on Tryptic Soy agar with 5% defibrinated sheep blood under propagation conditions.



## **Certificate of Analysis for NR-43529**

Date: 28 MAY 2015

Signature:

**BEI Resources Authentication** 

ATCC<sup>®</sup>, 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<sup>®</sup>'s knowledge.

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