## **Certificate of Analysis for NR-2713**

## Genomic DNA from Clostridium botulinum, Strain Walls 8G (VPI 4404)

## Catalog No. NR-2713

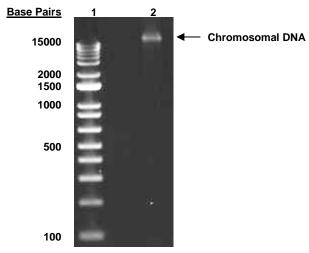
**Product Description:** Genomic DNA was isolated from a preparation of *Clostridium botulinum* (*C. botulinum*), strain Walls 8G (VPI 4404, BL 4851), serotype F (proteolytic).

Lot<sup>1</sup>: 70005630 Manufacturing Date: 17NOV2017

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1290 base pairs)	≥ 99% sequence identity to  C. botulinum, strain Walls 8G (GenBank: LBFH01000001.1)	100% sequence identity to  C. botulinum, strain Walls 8G (GenBank: LBFH01000001.1) <sup>2</sup>
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	0.4 μg in 50 μL per vial (7.5 μg/mL) <sup>3</sup>
Amount per vial	0.7 to 1.5 μg	0.4 μg²
Functional Activity by PCR Amplification 16S ribosomal RNA gene	~ 1500 base pair amplicon	~ 1500 base pair amplicon
OD <sub>260</sub> /OD <sub>280</sub> Ratio	1.7 to 2.1	2.0
Bacterial Inactivation 10% of total yield plated on agar for 14 days <sup>4</sup>	No viable bacteria detected	No viable bacteria detected

<sup>&</sup>lt;sup>1</sup>The bacterial preparation used for extraction of genomic DNA was produced from BEI Resources NR-272 lot 7364651. Genomic DNA was extracted using proprietary technology, and is provided in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH ~ 7.4).

Figure 1: Agarose Gel Electrophoresis



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

Lane 2: 75 ng of NR-2713

BEI Resources

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<sup>&</sup>lt;sup>2</sup>Also consistent with other species in the *C. sporogenes-C. botulinum* Group I clade; for more information, see Dobritsa, A. P., et al. "*Clostridium tepidum* sp. nov., a Close Relative of *Clostridium sporogenes* and *Clostridium botulinum* Group I." Int. J. Syst. Evol. Microbiol. 67 (2017): 2317-2322. PubMed: 28693684.

<sup>&</sup>lt;sup>3</sup>This result is below the specified concentration.

<sup>&</sup>lt;sup>4</sup>An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-positive and Gram-negative bacteria.



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/Heather Couch/ <u>Heather Couch</u>

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Program Manager or designee, ATCC Federal Solutions

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