

# **Certificate of Analysis for NR-51990**

### Enterovirus Species D Type 68, USA/2003-23226

### Catalog No. NR-51990

### **Product Description:**

Enterovirus species D type 68 (EV-D68), USA/2003-23226 was isolated in 2003 from a respiratory sample from a human subject who did not suffer from acute flaccid myelitis (AFM). NR-51990 lot 70040491 was produced by infecting rhabdomyosarcoma cells (RD; ATCC<sup>®</sup> CCL-136<sup>™</sup>) and incubating in Eagle's Minimum Essential Medium (ATCC<sup>®</sup> 30-2003<sup>™</sup>) supplemented with 2% fetal bovine serum (ATCC<sup>®</sup> 30-2020<sup>™</sup>) for 2 days at 33°C with 5% CO<sub>2</sub>.

### Passage History:

RD(5)/RD(2) (Prior to deposit at BEI Resources/BEI Resources); RD = rhabdomyosarcoma cells

Lot: 70040491 Manufacturing Date: 11DEC2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in RD Cells	Cell rounding and detachment	Cell rounding and detachment
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina <sup>®</sup> iSeq™ 100 Platform	≥ 98% identity with EV-D68, USA/2003-23226 (GenBank: MN240502.1)	99.9% identity with EV-D68, USA/2003-23226 (GenBank: MN240502.1)
Titer by TCID <sub>50</sub> Assay in RD Cells by Cytopathic Effect <sup>1</sup> (5 days at 33°C with 5% CO <sub>2</sub> )	Report results	2.8 × 10 <sup>6</sup> TCID <sub>50</sub> per mL
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>2</sup>	No growth	No growth
Trypticase Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C, aerobic	No growth	No growth
Mycoplasma Contamination		
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

<sup>&</sup>lt;sup>1</sup>The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation. <sup>2</sup>Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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Program Manager or designee, ATCC Federal Solutions

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