

# **Certificate of Analysis for NR-51995**

### Enterovirus Species D Type 68, USA/WI/2009-23230

### Catalog No. NR-51995

### **Product Description:**

Enterovirus species D type 68 (EV-D68), USA/WI/2009-23230 was isolated in 2009 from a respiratory sample from a human subject in Wisconsin, USA. The subject was not suffering from acute flaccid myelitis (AFM). NR-51995 lot 70041371 was produced by infecting rhabdomyosarcoma cells (RD; ATCC® CCL-136™) and incubating in Dulbecco's Modified Eagle's Medium (ATCC® 30-2002™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 2 days at 33°C with 5% CO₂.

#### Passage History:

RD(4)/RD(2) (Prior to deposit at BEI Resources/BEI Resources); RD = rhabdomyosarcoma cells

Lot: 70041371 Manufacturing Date: 11FEB2021

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in RD Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 850 nucleotides)	≥ 98% identity with EV-D68, USA/WI/2009-23230 (GenBank: MN240506.1)	99.9% identity with EV-D68, USA/WI/2009-23230 (GenBank: MN240506.1)
Titer by TCID₅ Assay in RD Cells by Cytopathic Effect¹ (7 days at 33°C with 5% CO₂)	Report results	8.9 × 10 <sup>7</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% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> 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% (LD<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> 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.

## /Heather Couch/

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