

**Enterovirus Species D Type 68, USA/2018-23089**

**Catalog No. NR-51998**

**Product Description:**

Enterovirus species D type 68 (EV-D68), USA/2018-23089 was isolated in 2018 from a nasopharyngeal swab of a human subject in the USA. The human subject was not suffering from acute flaccid myelitis. NR-51998 lot 70032011 was produced by infecting rhabdomyosarcoma cells (RD; ATCC® CCL-136™) and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 5 days at 33°C with 5% CO<sub>2</sub>.

**Passage History:**

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

**Lot: 70032011**

**Manufacturing Date: 21JAN2020**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in RD cells	Cell rounding and detachment	Cell rounding and detachment
Whole Genome Sequencing (~ 7290 nucleotides)	≥ 98% identity with EV-D68, USA/2018-23089 (GenBank: MK491182.1)	100% identity with EV-D68, USA/2018-23089 (GenBank: MK491182.1)
Titer by TCID <sub>50</sub> Assay in RD cells by Cytopathic Effect <sup>1</sup>	Report results	2.8 × 10 <sup>6</sup> TCID <sub>50</sub> per mL in 4 days at 33°C with 5% CO <sub>2</sub>
Amplification of EV-D68 Sequence by RT-PCR	~ 1100 base pair amplicon	~ 1100 base pair amplicon
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>2</sup> Trypticase Soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO <sub>2</sub>	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
<b>Mycoplasma Contamination</b> Agar and broth culture (14-day incubation at 37°C) DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	None detected None detected

<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. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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20 APR 2020

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