

**Enterovirus Species D Type 68, USA/2018-23206 (produced in Serum-Free A549 Cells)**

**Catalog No. NR-52355**

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**Product Description:**

Enterovirus species D type 68 (EV-D68), USA/2018-23206 was isolated in 2018 from a nasopharyngeal swab of a human subject in Maryland, USA. The human subject was suffering from acute flaccid myelitis. NR-52355 lot 70034955 was produced by infecting serum-free-adapted human lung carcinoma cells (A549; BEI Resources NR-52268) with the deposited material and incubating in PC-1™ Serum-Free Media (Lonza™ 344018) supplemented with 2% PC-1™ Supplement A (Lonza™ 344022) and 4 mM L-glutamine (ATCC® 30-2214™) for 4 days at 33°C and 5% CO<sub>2</sub>.

**Passage History:**

RD(5)/A(2) (Prior to deposit at BEI Resources/BEI Resources); RD = Rhabdomyosarcoma cells; A = Serum-free-adapted A549 cells

**Lot: 70034955**

**Manufacturing Date: 11MAY2020**

TEST	SPECIFICATIONS	RESULTS
<b>Identification by Infectivity in A549 Cells</b>	Cell rounding and detachment	Cell rounding and detachment
<b>Titer by TCID<sub>50</sub> Assay in A549 Cells by Cytopathic Effect<sup>1</sup></b> (9 days at 33°C and 5% CO <sub>2</sub> )	Report results	2.8 × 10 <sup>7</sup> TCID <sub>50</sub> per mL
<b>Amplification of EV-D68 Sequence by RT-PCR</b>	~ 1100 base pair amplicon	~ 1100 base pair amplicon
<b>Sequencing of Species-Specific Region</b> (~ 1010 nucleotides)	≥ 98% identity with EV-D68, USA/2018-23206 (GenBank: MN389731.1)	99.8% identity with EV-D68, USA/2018-23206 (GenBank: MN389731.1)
<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, aerobic	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.

/Sonia Bjorum Brower/

Sonia Bjorum Brower

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Lead Technical Writer or designee, ATCC Federal Solutions

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