SUPPORTING INFECTIOUS DISEASE RESEARCH

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

### Catalog No. NR-52355

This reagent is the property of the U.S. Government.

#### **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<sup>™</sup> Serum-Free Media (Lonza<sup>™</sup> 344018) supplemented with 2% PC-1<sup>™</sup> Supplement A (Lonza<sup>™</sup> 344022) and 4 mM L-glutamine (ATCC<sup>®</sup> 30-2214<sup>™</sup>) 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-freeadapted A549 cells

## Lot: 70034955

## Manufacturing Date: 11MAY2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in A549 Cells	Cell rounding and detachment	Cell rounding and detachment
Titer by TCID <sub>50</sub> Assay in A549 Cells by Cytopathic Effect <sup>1</sup> (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
Amplification of EV-D68 Sequence by RT-PCR	~ 1100 base pair amplicon	~ 1100 base pair amplicon
Sequencing of Species-Specific Region (~ 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)
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

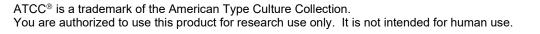
<sup>1</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 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.

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