

Enterovirus Species D Type 68, USA/2018-23209 (produced in serum-free A549 cells)

Catalog No. NR-52356

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Product Description:

Enterovirus species D type 68 (EV-D68), USA/2018-23209 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-52356 lot 70035462 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 2 days at 33°C and 5% CO₂.

Passage History:

RD(3)/A(2) (Prior to deposit at BEI Resources/BEI Resources); RD = Rhabdomyosarcoma cells; A = Serum-free-adapted A549 cells

Lot: 70035462

Manufacturing Date: 15MAY2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in A549 Cells	Cell rounding and detachment	Cell rounding and detachment
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with EV-D68, USA/2018-23209 (GenBank: MN246002.1) ≥ 98% identity with EV-D68, USA/2018-23209 (GenBank: MN389732.1)	99.92% identity with EV-D68, USA/2018-23209 (GenBank: MN246002.1) 99.96% identity with EV-D68, USA/2018-23209 (GenBank: MN389732.1)
Sequencing of Species-Specific Region (~ 800 nucleotides)	≥ 98% identity with EV-D68, USA/2018-23209 (GenBank: MN246002.1) ≥ 98% identity with EV-D68, USA/2018-23209 (GenBank: MN389732.1)	99.6% identity with EV-D68, USA/2018-23209 (GenBank: MN246002.1) 99.6% identity with EV-D68, USA/2018-23209 (GenBank: MN389732.1)
Titer by TCID ₅₀ Assay in A549 Cells by Cytopathic Effect ¹ (6 days at 33°C and 5% CO ₂)	Report results	2.8 × 10 ⁷ TCID ₅₀ per mL
Amplification of EV-D68 Sequence by RT-PCR	~ 1100 base pair amplicon	~ 1100 base pair amplicon
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ² 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
Mycoplasma Contamination 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

¹The 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.

²Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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10 SEP 2020

Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-52356 lot 70035462

Sequence analysis resulted in the discovery of seven SNPs when compared to reference sequence GenBank MN246002.1 (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52356_70035462 Sequence	Position in MN246002.1	Reported MN246002.1 Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
623	625	G	A	221	SNP	1	0.7229627
1148	1150	C	T	228	SNP	1	0.9984067
2477	2479	C	T	228	SNP	1	0.9989958
2769	2771	T	A	221	SNP	1	0.7527854
2780	2782	C	A	221	SNP	1	0.7667686
5188	5190	A	G	225	SNP	1	1.0000000
7310	7312	T	C	113	SNP	1	1.0000000

Sequence analysis resulted in the discovery of three SNPs when compared to reference sequence GenBank MN389732.1 (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52356_70035462 Sequence	Position in MN389732.1	Reported MN389732.1 Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
623	633	G	A	221	SNP	1	0.7229627
2769	2779	T	A	221	SNP	1	0.7526272
2780	2790	C	A	221	SNP	1	0.7666123