

Enterovirus Species D Type 68, USA/2018-23088 (produced in serum-free A549 cells)

Catalog No. NR-52016

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Product Description:

Enterovirus species D type 68 (EV-D68), USA/2018-23088 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-52016 lot 70032743 was produced by infecting serum-free adapted human lung carcinoma cells (A549; BEI Resources NR-52268) and incubating in PC-1™ serum-free medium (Lonza™ 344018) containing 2% PC-1™ medium supplement (Lonza™ 344022) and 4 mM L-glutamine (ATCC® 30-2214™) for 6 days at 33°C with 5% CO₂.

Passage History:

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

Lot: 70032743

Manufacturing Date: 12MAR2020

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/2018-23088 (GenBank: MK491181.1)	99.92% identity with EV-D68, USA/2018-23088 (GenBank: MK491181.1)
Sequencing of Species-Specific Region (~ 1010 nucleotides)	≥ 98% identity with EV-D68, USA/2018-23088 (GenBank: MK491181.1)	99.7% identity with EV-D68, USA/2018-23088 (GenBank: MK491181.1)
Titer by TCID₅₀ Assay in A549 Cells by Cytopathic Effect¹ (8 days at 33°C with 5% CO ₂)	Report results	8.9 × 10 ⁶ 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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25 SEP 2020

Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-52016 lot 70032743

Sequence analysis resulted in the discovery of four SNPs when compared to reference sequence GenBank MK491181.1 (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52016_70032743 Sequence	Position in MK491181.1	Reported MK491181.1 Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
965	966	G	A	222	SNP	1	0.4716806
2108	2109	C	T	222	SNP	1	0.4916174
2634	2635	G	A	222	SNP	1	0.4703501
3189	3190	A	G	222	SNP	1	0.6499443
3191	3192	G	A	222	SNP	1	0.4096139
5994	5995	T	C	222	SNP	1	0.3797294