

Certificate of Analysis for NR-51997

Enterovirus Species D Type 68, USA/2018-23088

Catalog No. NR-51997

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-51997 lot 70032009 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 3 days at 33°C with 5% CO₂.

Passage History:

RD(5)/RD(2) (Prior to deposit at BEI Resources/BEI Resources); RD = Rhabdomyosarcoma cells

Lot: 70032009 Manufacturing Date: 11JAN2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in RD cells	Cell rounding and detachment	Cell rounding and detachment
Whole Genome Sequencing (~ 7220 nucleotides)	≥ 98% identity with EV-D68, USA/2018-23088 (GenBank: MK491181.1)	100% identity with EV-D68, USA/2018-23088 (GenBank: MK491181.1)
Titer by TCID ₅₀ Assay in RD cells by Cytopathic Effect ¹	Report results	8.9×10^6 TCID ₅₀ per mL in 6 days at 33°C with 5% CO ₂
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	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 and 5% CO ₂	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

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

/Heather Couch/

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Program Manager or designee, ATCC Federal Solutions

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