

Enterovirus Species A Type 71, USA/WA/2016-19522

Catalog No. NR-51999

Product Description:

Enterovirus species A type 71 (EV-A71), USA/WA/2016-19522 was isolated in 2016 from the stool sample of a human subject suffering from acute flaccid myelitis in the USA. NR-51999 lot 70032013 was produced by infecting human lung fibroblast cells (HLF-a; ATCC® CCL-199™) and incubating in Eagle’s Minimum Essential Medium (ATCC® 30-2003) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 4 days at 37°C with 5% CO₂.

Passage History:

RD(5),HLF(3)/HLF(2) (Prior to deposit at BEI Resources/BEI Resources); RD = Rhabdomyosarcoma cells; HLF = Human lung fibroblast (HLF-a) cells

Lot: 70032013

Manufacturing Date: 27JAN2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in HLF-a Cells	Cell rounding and detachment	Cell rounding and detachment
Whole Genome Sequencing (~ 7210 nucleotides)	≥ 98% identity with EV-A71, USA/WA/2016-19522 (GenBank: KY888026.1)	99.9% identity with EV-A71, USA/WA/2016-19522 (GenBank: KY888026.1)
Titer by TCID₅₀ Assay in HLF-a Cells by Cytopathic Effect¹ (8 days at 37°C with 5% CO ₂)	Report results	2.8 × 10 ⁶ TCID ₅₀ per mL
Amplification of EV-A71 Sequence by RT-PCR	~ 840 base pair amplicon	~ 840 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 and 5% CO ₂	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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