

Enterovirus Species D Type 68, US/KY/14-18953 (produced in serum-free A549 cells)

Catalog No. NR-52014

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Product Description:

Enterovirus species D type 68 (EV-D68), US/KY/14-18953 was isolated in August 2014 from a nasopharyngeal swab taken from a human in Kentucky, USA. NR-52014 lot 70032739 was produced by infecting serum-free-adapted human lung carcinoma cells (A549; BEI Resources NR-52268) with BEI Resources seed 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 3 days at 33°C and 5% CO₂.

Passage History:

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

Lot: 70032739

Manufacturing Date: 09MAR2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in A549 cells	Cell rounding and detachment	Cell rounding and detachment
Whole Genome Sequencing (~ 7330 nucleotides)	≥ 98% identity with EV-D68, US/KY/14-18953 (GenBank: KM851231.1)	99.9% identity with EV-D68, US/KY/14-18953 (GenBank: KM851231.1)
Sequencing of Species-Specific Region (~ 970 nucleotides)	≥ 98% identity with EV-D68, US/KY/14-18953 (GenBank: KM851231.1)	99.8% identity with EV-D68, US/KY/14-18953 (GenBank: KM851231.1)
Titer by TCID₅₀ Assay in A549 cells by Cytopathic Effect¹ (7 days at 33°C and 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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