b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-51994

Enterovirus Species D Type 68, USA/MD/2009-23229

Catalog No. NR-51994

Product Description:

Enterovirus species D type 68 (EV-D68), USA/MD/2009-23229 was isolated in 2009 from a respiratory sample from a human subject in Maryland, USA. The subject was not suffering from acute flaccid myelitis (AFM). NR-51994 lot 70041369 was produced by infecting rhabdomyosarcoma cells (RD; ATCC® CCL-136™) and incubating in Dulbecco's Modified Eagle's Medium (ATCC® 30-2002™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 3 days at 33°C with 5% CO_2.

Passage History:

RD(4)/RD(2) (Prior to deposit at BEI Resources/BEI Resources); RD = rhabdomyosarcoma cells

Lot: 70041369 **Manufacturing Date: 12FEB2021**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in RD Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 840 nucleotides)	≥ 98% identity with EV-D68, USA/MD/2009-23229 (GenBank: MN240505.1)	99.3% identity with EV-D68, USA/MD/2009-23229 (GenBank: MN240505.1)
Titer by $TCID_{50}$ Assay in RD Cells by Cytopathic Effect[1] (7 days at 33°C with 5% CO_2)	Report results	8.9×10^7 $TCID_{50}$ per mL
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, aerobic	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

[1]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.

[2]Atlas, Ronald M. Handbook of Microbiological Media. 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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