

Certificate of Analysis for NR-55941

Enterovirus Species D Type 68, USA/2020-23336

Catalog No. NR-55941

This reagent is the tangible property of the U.S. Government.

Product Description:

Enterovirus D68 (EVD-68), USA/2020/23336 was isolated from a human respiratory sample. The patient's AFM status is not known. NR-55941 lot 70056901 was produced by infecting Rhabdomyosarcoma cells (RD; ATCC® CCL-136™) with the deposited material 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 and passaged once for another 3 days at 33°C with 5% CO₂.

Passage History:

RD(3)/RD(2) (Centers for Disease Control and Prevention/BEI Resources); RD = Rhabdomyosarcoma cells

Lot: 70056901 Manufacturing Date: 12DEC2022

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in RD Cells	Cell rounding and detachment	Cell rounding and detachment
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina [®] iSeq™ 100 Platform	≥ 98% identity with EV-D68, (GenBank: MN726801)	99.28% identity with EV-D68, (GenBank: MN726801)
Titer by TCID ₅₀ Assay in RD Cells by Cytopathic Effect ¹ (8 days at 33°C with 5% CO ₂)	Report results	1.6 × 10 ⁷ TCID ₅₀ /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

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.

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Technical Manager or designee, ATCC Federal Solutions

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