

Certificate of Analysis for NR-55939

Enterovirus Species D Type 68, USA/2020-23334

Catalog No. NR-55939

Product Description:

Enterovirus species D type 68 (EV-D68), USA/2020-23334 was isolated in 2020 from a respiratory sample from a human subject in the USA. The patient's acute flaccid myelitis status is not known. NR-55939 lot 70056897 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: 70056897 Manufacturing Date: 16DEC2022

| 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 EVD-68, (GenBank: MN726801) | 99.3% identity with EVD-68, (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 | 2.8 × 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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