

# **Certificate of Analysis for NR-52000**

#### Enterovirus Species A Type 71, USA/2018-23092

## Catalog No. NR-52000

## **Product Description:**

Enterovirus species A type 71 (EV-A71), USA/2018-23092 was isolated in 2018 from the stool sample of a human subject suffering from acute flaccid myelitis in the USA. NR-52000 lot 70032015 was produced by infecting rhabdomyosarcoma cells (RD; ATCC® CCL-136™) 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(3)/RD(2) (Prior to deposit at BEI Resources/BEI Resources); RD = Rhabdomyosarcoma cells

Lot: 70032015 Manufacturing Date: 20JAN2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in RD cells	Cell rounding and detachment	Cell rounding and detachment
Whole Genome Sequencing (~ 7400 nucleotides)	≥ 98% identity with EV-A71, USA/2018-23092 (GenBank: MK652139.1)	100% identity with EV-A71, USA/2018-23092 (GenBank: MK652139.1)
Titer by TCID <sub>50</sub> Assay in RD cells by Indirect Fluorescent Antibody (IFA) Readout <sup>1,2</sup> 7 days at 37°C with 5% CO <sub>2</sub>	Report results	1.6 × 10 <sup>7</sup> TCID <sub>50</sub> per mL
Amplification of EV-A71 Sequence by RT-PCR	~ 890 base pair amplicon	~ 890 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>3</sup>	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 and 5% CO <sub>2</sub>	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. ²Using Pan-Enterovirus Reagent (Light Diagnostics™ 3360)

#### /Heather Couch/

Heather Couch 23 APR 2020

Program Manager or designee, ATCC Federal Solutions

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<sup>&</sup>lt;sup>3</sup>Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.