

**Enterovirus D68, US/IL/14-18956**

**Catalog No. NR-49133**

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**Product Description:** Enterovirus D68 (EV-D68), US/IL/14-18956 was isolated in September 2014 from a nasopharyngeal swab taken from a human in Illinois, USA.

**Passage History:** RD4/RD1 (Prior to deposit at BEI Resources/BEI Resources); RD = RD cells<sup>1</sup>

**Lot<sup>1,2</sup>: 70018975**

**Manufacturing Date: 05OCT2018**

TEST	SPECIFICATIONS	RESULTS
<b>Identification by Infectivity in RD cells<sup>1</sup></b>	Cell rounding and detachment	Cell rounding and detachment
<b>Sequencing of Species-Specific Region</b> (~ 980 nucleotides)	≥ 98% identity with EV-D68, US/IL/14-18956 (GenBank: MK268345.1)	99.9% identity with EV-D68, US/IL/14-18956 (GenBank: MK268345.1)
<b>Titer by TCID<sub>50</sub> Assay<sup>3,4</sup> in RD cells<sup>1</sup> by Cytopathic Effect</b>	Report results	1.1 × 10 <sup>8</sup> TCID <sub>50</sub> per mL
<b>Amplification of EV-D68 Sequence by RT-PCR</b>	~ 1100 base pair amplicon	~ 1100 base pair amplicon
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>5</sup> , 37°C and 26°C, aerobic Trypticase Soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Blood agar, 37°C, aerobic Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO <sub>2</sub>	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
<b>Mycoplasma Contamination</b> 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

<sup>1</sup>Human rhabdomyosarcoma cells (RD cells; ATCC® CCL-136™)

<sup>2</sup>Lot 70018975 of NR-49133 was produced by infecting RD cells<sup>1</sup> with BEI Resources NR-49133 lot 63264128 and incubating in Eagle's Minimum Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate and 1.5 g/L of sodium bicarbonate (ATCC® 30-2003) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 3 days at 33°C with 5% CO<sub>2</sub>.

<sup>3</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> 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<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> provides a measure of the titer (or infectivity) of a virus preparation.

<sup>4</sup>The culture was incubated for 5 days at 33°C and 5% CO<sub>2</sub>.

<sup>5</sup>Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798

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