

## Certificate of Analysis for NR-49133

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

Catalog No. NR-49133

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

**Product Description:** Cell lysate and supernatant from human rhabdomyosarcoma cells<sup>1</sup> infected

with Enterovirus D68, US/IL/14-18956

Passage History: RD4 (RD# = Number of passages in RD cells)

Lot<sup>2,3</sup>: 63264128 Manufacturing Date: 03OCT2014

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in RD Cells <sup>1</sup>	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (1000 nucleotides)	Consistent with Enterovirus D68	Consistent with Enterovirus D68 <sup>4</sup>
Titer by TCID <sub>50</sub> Assay <sup>5,6</sup> in RD Cells <sup>1</sup>	Report results	2.8 × 10 <sup>8</sup> TCID <sub>50</sub> per mL
Sterility (21-day incubation) Harpo's HTYE broth <sup>7</sup> , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO <sub>2</sub>	No growth	None detected
Mycoplasma Contamination  Agar and broth culture (14-day incubation at 37°C)  DNA Detection by PCR of Test Article nucleic acid	None detected None detected	None detected None detected

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

**Date:** 15 JUL 2015

Signature:

**BEI Resources Authentication** 

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

BEI Resources www.beiresources.org E-mail: <a href="mailto:contact@beiresources.org">contact@beiresources.org</a>
Tel: 800-359-7370

Fax: 703-365-2898

<sup>&</sup>lt;sup>2</sup>Produced at the Poliovirus and Picornavirus Laboratory Branch, Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

<sup>&</sup>lt;sup>3</sup>Grown in Eagle's Minimum Essential Medium at 33°C and 5% CO<sub>2</sub>

<sup>&</sup>lt;sup>4</sup>Sequence information for Enterovirus D68, US/IL/14-18956 is not available in the NCBI database. The nucleotide sequence obtained for NR-49133, Lot 63264128 matched a partial genomic sequence provided by the depositor with 99.9% identity, and was consistent with other strains isolated during the 2014 outbreak.

<sup>&</sup>lt;sup>5</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>64</sup> days at 33°C and 5% CO2

<sup>&</sup>lt;sup>7</sup>Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.