

# Certificate of Analysis for NR-49138

### Genomic RNA from Enterovirus D68, US/IL/14-18956

#### Catalog No. NR-49138

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

#### **Product Description:**

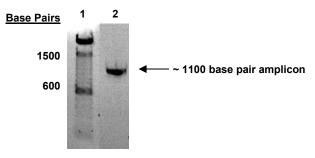
Genomic RNA was isolated from a preparation of cell lysate and supernatant from human rhabdomyosarcoma (RD) cells infected with enterovirus D68 (EV-D68), US/IL/14-18956.1

Lot: 70026007<sup>2</sup> Manufacturing Date: 05JUN2019

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis Sequencing of VP1 Capsid Gene (~ 960 nucleotides)	≥ 99% sequence identity to EV D68, US/IL/14-18956 (GenBank: MK268345.1)	99.9% sequence identity to EV D68, US/IL/14-18956 (GenBank: MK268345.1)
Functional Activity by RT-PCR Amplification <sup>3</sup> VP1 capsid protein gene Pre-Vial Concentration by RiboGreen® Measurement (Viral, Cellular and Carrier) <sup>4</sup>	~ 1100 base pair amplicon  Report results	~ 1100 base pair amplicon (Figure 1) 3.0 ng in 100 μL per vial (0.03 μg/mL)
Estimated Amount per Vial <sup>4</sup>	Report Results	3.0 ng
Virus Inactivation  10% of total yield inoculated on RD cells and evaluated for cytopathic effect (CPE) and viral protein expression by immunofluorescence assay (IFA) <sup>1,5,6</sup>	No viable virus detected	No viable virus detected

¹RD cells: ATCC® CCL-136™

Figure 1: Functional Activity of NR-49138 by RT-PCR Amplification of VP1 Capsid Protein Gene



Lane 1: Invitrogen™ 100 bp DNA Ladder Lane 2: PCR product from 0.2 µL of NR-49138

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<sup>&</sup>lt;sup>2</sup>Nucleic acid was extracted from a preparation of enterovirus D68, US/IL/14-18956 (BEI Resources NR-49133 lot 70018975), using a QIAamp<sup>®</sup> Viral RNA Mini Kit (Qiagen 52906).

³cDNA was generated using iScript<sup>τM</sup> cDNA Synthesis kit (Bio-Rad 170-8891). 2 μL of NR-49138 cDNA was amplified in a 50 μL reaction using iTaq<sup>™</sup> DNA Polymerase (Bio-Rad 170-8870).

<sup>&</sup>lt;sup>4</sup>Measurement is determined pre-vial prior to dilution due to the limit of detection of the quantification method.

<sup>&</sup>lt;sup>5</sup>Use of the QIAamp<sup>®</sup> Viral RNA Mini Kit has been demonstrated to consistently inactivate enterovirus as shown by the absence of CPE and viral protein expression by IFA after plating the entire extract on virus-susceptible cells.

<sup>&</sup>lt;sup>6</sup>IFA performed using Pan-Enterovirus Reagent (Millipore 3360)



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/Heather Couch/ Heather Couch

04 SEP 2019

Program Manager or designee, ATCC Federal Solutions

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