

Certificate of Analysis for NR-49132

Enterovirus D68, US/KY/14-18953

Catalog No. NR-49132

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

Product Description: Cell lysate and supernatant from human rhabdomyosarcoma cells¹ infected with Enterovirus D68, US/KY/14-18953

Passage History: RD3/RD2 (Centers for Disease Control and Prevention/BEI Resources; RD# = Number of passages in RD cells)

Lot²: 63205992 Manufacturing Date: 02JAN2015

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in RD Cells ¹	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (870 nucleotides)	Consistent with Enterovirus D68, US/KY/14-18953	99% identity with Enterovirus D68, US/KY/14-18953 (GenBank: KM851231)
Titer by TCID ₅₀ Assay ^{3,4} in RD Cells ¹	Report results	1.6 × 10 ⁸ TCID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth ⁵ , 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 ₂	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

¹RD cells (ATCC[®] CCL-136™)

Date: 24 MAR 2015

Signature

Title:

Technical Manager, BEI Authentication or designee

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.

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²Grown in Eagle's Minimum Essential Medium (ATCC® 30-2003™) for 3 days at 33°C and 5% CO₂

³The 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.

⁴5 days at 33°C and 5% CO₂

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