

**Enterovirus Species D Type 94, ANG/2010-23293**

**Catalog No. NR-52317**

**Product Description:**

Enterovirus species D type 94 (EV-D94), ANG/2010-23293 was isolated in 2010 from a stool specimen from a human subject in Angola. The subject was not suffering from acute flaccid myelitis (AFM). NR-52317 lot 70043452 was produced by infecting rhabdomyosarcoma cells (RD; ATCC® CCL-136™) and incubating in Dulbecco's Modified Eagle's Medium (ATCC® 30-2002™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 8 days at 37°C with 5% CO<sub>2</sub>.

**Passage History:**

RD(3)/RD(2) (Prior to deposit at BEI Resources/BEI Resources); RD = rhabdomyosarcoma cells

**Lot: 70043452**

**Manufacturing Date: 28APR2021**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in RD Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 840 nucleotides)	≥ 98% identity with EV-D94, ANG/2010-23293 (GenBank: MT081370.1)	100% identity with EV-D94, ANG/2010-23293 (GenBank: MT081370.1)
Titer by TCID <sub>50</sub> Assay in RD Cells by Indirect Fluorescent Antibody <sup>1,2</sup> (12 days at 37°C with 5% CO <sub>2</sub> )	Report results	1.8 × 10 <sup>4</sup> TCID <sub>50</sub> per mL <sup>3</sup>
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>4</sup> 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, aerobic	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
Mycoplasma Contamination 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>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>2</sup>Using Pan-enterovirus (Millipore 3360)

<sup>3</sup>Titer was determined by indirect fluorescent antibody (IFA) and completed in duplicate (2.81 × 10<sup>4</sup> per mL and 8.89 × 10<sup>3</sup> per mL). The average of the two values is reported.

<sup>4</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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14 DEC 2021

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