

Certificate of Analysis for NR-49914

Mayaro Virus, Uruma

Catalog No. NR-49914

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero)¹ infected with Mayaro virus, Uruma

Passage History: XV1/V2 (Prior to deposit at BEI Resources/BEI Resources); X = unknown, V# = Number of passages in Vero cells²

Lot³: 63856762 Manufacturing Date: 15JAN2016

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells ¹	Cell rounding and detachment	Cell rounding and detachment
Whole Genome Sequencing (11430 nucleotides)	Consistent with Mayaro virus	Consistent with Mayaro virus ⁴
Titer by TCID ₅₀ Assay ^{5,6} in Vero Cells ¹	Report results	1.6 × 10 ⁸ TCID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth ⁷ , 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	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

¹Vero cells: ATCC® CCL-81™

Date: 13 JUL 2016

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.

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²The first virus passage at BEI Resources was performed by lipofectamine transfection of extracted viral nucleic acid in order to remove contaminating mycoplasma.

³Grown 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 2 days at 37°C with 5% CO₂.

⁴The complete genomic sequence obtained for NR-49914 at BEI Resources has >99% identity with the genome of Mayaro virus, MAYLC (GenBank: DQ001069) and also has >99% identity with the limited sequence available for Mayaro virus, Uruma (GenBank: DQ138318 and DQ487395).

⁵The 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.

⁶7 days at 37°C and 5% CO₂

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