

Punta Toro Virus, GML488778

Catalog No. NR-49730

Product Description:

Punta Toro virus (PTV), GML488778 was isolated from a human (*Homo sapiens*) in Panama in January 2004. In order to remove contaminating mycoplasma, NR-49730 was passaged three times in the presence of mycoplasma elimination reagent (Plasmocin™; InvivoGen® ant-MPP). NR-49730 lot 70027646 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells (Vero E6; ATCC® CRL-1586™) with mycoplasma-cured depositor material and incubating in Eagle's Minimum Essential Medium (ATCC 30-2003™) supplemented with 2% fetal bovine serum (ATCC 30-2020™) for 5 days at 37°C with 5% CO₂.

Passage History:

V(2)/VE(5) (Prior to deposit at BEI Resources/BEI Resources); V = Vero cells; VE = Vero E6 cells

Lot: 70027646

Manufacturing Date: 27JAN2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 910 nucleotides)	≥ 98% identity with PTV, GML488778 (GenBank: KP272037.1)	99.9% identity with PTV, GML488778 (GenBank: KP272037.1)
Titer by TCID ₅₀ Assay in Vero E6 Cells by Cytopathic Effect ¹ (7 days at 37°C with 5% CO ₂)	Report results	1.6 × 10 ⁷ TCID ₅₀ per mL
Amplification of PTV Sequence by RT-PCR	~ 970 base pair amplicon	~ 970 base pair amplicon
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ² 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

¹The 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.

²Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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