

Certificate of Analysis for NR-17597

Kern Canyon Virus, M-206

Catalog No. NR-17597

Product Description:

Kern Canyon virus (KCV), M-206 was isolated from a mouse-eared bat (*Myotis yumanensis*) in Kern Canyon, California in 1956. In order to remove contaminating mycoplasma, the deposited material was passaged three times in mycoplasma removal agent (Plasmocin; InvivoGen ant-MPP). NR-17597 lot 70026757 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells (Vero E6; ATCC[®] CRL-1586™) with the treated material and incubating in Eagle's Minimum Essential Medium (ATCC[®] 30-2003) supplemented with 2% fetal bovine serum (ATCC[®] 30-2020) for 7 days at 37°C with 5% CO₂.

Passage History:

X(?)/VE(4) (Prior to deposit at BEI Resources/BEI Resources); X = Unknown; VE = Vero E6 cells

Lot: 70026757 Manufacturing Date: 03OCT2019

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 920 nucleotides)	≥ 98% identity with KCV	99.9% identity with KCV, strain M03790 (GenBank: KM204992.1) ¹
Titer by TCID ₅₀ Assay in Vero E6 Cells by Cytopathic Effect ² (6 days at 37°C with 5% CO ₂)	Report results	8.9 × 10 ⁸ TCID ₅₀ per mL
Amplification of KCV Sequence by RT-PCR	~ 1000 base pair amplicon	~ 1000 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic ³	No growth	No growth
Trypticase Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
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)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

Sequence information for KCV, M-206 is not available in the NCBI database; nucleotide sequence obtained for NR-17597 lot 70026757 is ≥ 98% identical to KCV, strain M03790 which is contemporary with strain M-206 and was also collected from a bat in the USA in 1956 (see Walker, P. J., et al. "Evolution of Complexity and Genome Size in the *Rhabdoviridae*." PLoS Pathog. 11 (2015): e1004664. PubMed: 25679389).

/Heather Couch/

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Program Manager or designee, ATCC Federal Solutions

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²The Tissue Culture Infectious Ďose 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.
³Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.