

Certificate of Analysis for NR-22243

Human Metapneumovirus, TN/96-213

Catalog No. NR-22243

Product Description:

Human metapneumovirus (HMPV), TN/96-213 was isolated from a human specimen collected in Tennessee, USA, in 1996. NR-22243 lot 70062308 was produced by infecting *Macaca mulatta* kidney cells (LLC-MK2; ATCC[®] CCL-7.1[™]) in Opti-MEM[®] Minimal Essential Medium (Gibco[®] 31985) supplemented with, 2 mM L-glutamine (ATCC[®] 30-2214[™]), 100 μg/mL CaCl₂, and 5 μg/mL trypsin (ATCC[®] 30-2101[™]) for 7 days at 37°C and 5% CO₂.

Passage History:

L(3)/L(5) (Vanderbilt/BEI Resources); L = LLC-MK2 cells

Lot: 70062308 Manufacturing Date: 25AUG2023

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TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in LLC-MK2 Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region Glycoprotein gene (~ 280 nucleotides)	≥ 98% identity with HMPV, TN96-213 glycoprotein gene, complete cds (GenBank: JF929885.1)	99.6% identity with HMPV, TN96- 213 glycoprotein gene, complete cds (GenBank: JF929885.1) ¹
Glycoprotein gene (~ 650 nucleotides)	≥ 98% identity with HMPV/USA/TN-96- 213/1996/B complete genome (GenBank: KC562229.1)	98.0% identity with HMPV/USA/TN-96-213/1996/B complete genome (GenBank: KC562229.1) ¹
Titer by TCID ₅₀ Assay in LLC-MK2 Cells by RT-PCR ² (9 days at 35°C with 5% CO ₂)	Report results	8.9 × 10 ⁵ TCID ₅₀ /mL
Amplification of HMPV Sequence by RT-PCR	~ 1300 base pair amplicon	~ 1300 base pair amplicon
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic³ 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
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

The reason for the discrepancy between the two published sequences [HMPV, TN/96-213 complete genome (GenBank: KC562229.1) and HMPV, isolate TN96-213 glycoprotein, complete cds (GenBank: JF929885.1) is unclear. Please see Yang, C. F., et al. "Human Metapneumovirus G Protein is Highly Conserved within but not between Genetic Lineages." <u>Arch. Virol.</u> 158 (2013): 1245-1252. PubMed: 23385328)] for additional information.
 The 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
 Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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Technical Manager or designee, ATCC Federal Solutions

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