

Certificate of Analysis for NR-22232

Human Metapneumovirus, TN/91-316

Catalog No. NR-22232

Product Description:

Note: The organism name on the vial label for lot 70040318 is incorrect. The correct name is human metapneumovirus. Human metapneumovirus (HMPV), TN/91-316 was isolated in 1991 from a human specimen collected in Tennessee, USA. NR-22232 lot 70040318 was produced by infecting *Macaca mulatta* kidney epithelial 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 per mL CaCl₂ and 5 µg per mL trypsin (ATCC[®] 30-2101™) for 7 days at 37°C and 5% CO₂.

Passage History:

L8/L6 (Prior to BEI Resources/BEI Resources); L = LLC-MK2 cells

Lot: 70040318 Manufacturing Date: 18NOV2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in LLC-MK2 Derivative Cells	Report results	Cell rounding and sloughing
Identification by Direct Fluorescent Antibody (DFA) Assay ¹	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region ² (890 nucleotides)	≥ 99% identity with HMPV, TN/91-316 (GenBank: KC403971.1)	100% identity with HMPV, TN/91-316 (GenBank: KC403971.1)
Titer by TCID ₅₀ Assay in LLC-MK2 Cells with DFA Readout ^{1,3} (14 days at 37°C and 5% CO ₂)	Report results	2.8 × 10 ⁶ TCID ₅₀ per mL
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic ⁴	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, 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

¹Using human metapneumovirus DFA reagent (MilliporeSigma® 5091RUO)

/Heather Couch/

Heather Couch 08 MAR 2021

Program Manager or designee, ATCC Federal Solutions

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 limited nucleotide sequencing of NR-22232 performed at BEI Resources is not sufficient to confirm exact strain identity owing to the high degree of sequence conservation within HMPV lineages.

³The 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.
⁴Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.