

Certificate of Analysis for NR-22232

Human Metapneumovirus, TN/91-316

Catalog No. NR-22232

Product Description: Human metapneumovirus (HMPV), TN/91-316 was isolated in 1991 from a human specimen collected in Tennessee, USA. Each vial contains cell lysate and supernatant from Macaca mulatta kidney epithelial cells¹ infected with HMPV, TN/91-316.

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

Lot²: 70005955 Manufacturing Date: 29JUN2017

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in LLC-MK2 Derivative Cells ¹	Report results	Rounding and dissociation from the monolayer
Sequencing of Species-Specific Region ³ (~ 890 nucleotides)	Consistent with HMPV, TN/91-316	100% identity with HMPV, TN/91-316 (GenBank: KC403971.1)
Titer by TCID ₅₀ Assay ^{4,5} in LLC-MK2 Derivative Cells ¹ with Direct Fluorescence Assay (DFA) Readout ⁶	Report results	1.6 × 10 ⁷ 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 Test Article nucleic acid	None detected None detected	None detected None detected

¹LLC-MK2 Derivative cells (ATCC[®] CCL-7.1 ™)

/Heather Couch/

Heather Couch 18 JUN 2018

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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BEI Resources

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²Grown in Opti-MEM® Minimal Essential Medium (Life Technologies 31985) supplemented with 2 mM L-glutamine (ATCC® 30-2214), 100 µg per mL CaCl₂ (Fisher BioReagents™ BP9742), and 5 μg per mL trypsin (ATCC® 30-2101) for 7 days at 37°C and 5% CO₂

³The 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.

⁴The 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

⁵¹³ days at 37°C and 5% CO₂

⁶Using Light Diagnostics™ Human Metapneumovirus DFA Reagent (Millipore 5091)

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