

# Certificate of Analysis for NR-22240

### Human Metapneumovirus, TN/93-32

### Catalog No. NR-22240

### **Product Description:**

Human metapneumovirus (HMPV), TN/93-32 was isolated in 1993 from a human specimen collected in Tennessee, USA. NR-22240 lot 70039860 was produced by infecting *Macaca mulatta* kidney epithelial cells (LLC-MK2; ATCC<sup>®</sup> CCL-7.1™) in Opti-MEM<sup>®</sup> Minimal Essential Medium (Gibco<sup>®</sup> 31985) supplemented with 2 mM L-glutamine (ATCC<sup>®</sup> 30-2214™), 100 μg per mL CaCl<sub>2</sub>, and 5 μg per mL trypsin (ATCC<sup>®</sup> 30-2101™) for 5 days at 37°C and 5% CO<sub>2</sub>.

### Passage History:

L(X)/L(7) (Vanderbilt/BEI Resources); L = LLC-MK2 cells; X = unknown

Lot: 70039860 Manufacturing Date: 24NOV2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in LLC-MK2 Derivative Cells	Report results	Cell rounding and sloughing
Identification by Direct Fluorescent Antibody (DFA) Assay <sup>1</sup>	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region <sup>2</sup> Gene L (890 nucleotides)	≥ 99% identity with HMPV, TN/93-32 (GenBank: KC562223.1)	99.9% identity with HMPV, TN/93-32 (GenBank: KC562223.1)
Titer by TCID <sub>50</sub> Assay in LLC-MK2 Cells with DFA Readout <sup>1,3</sup> (7 days at 37°C and 5% CO <sub>2</sub> )	Report results	1.6 × 10 <sup>6</sup> TCID <sub>50</sub> per mL
Amplification of HMPV Sequence by RT-PCR	~ 980 base pair amplicon	~ 980 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>4</sup>	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

<sup>&</sup>lt;sup>1</sup>Using FITC-labeled human metapneumovirus DFA reagent (Light Diagnostics™ 5091)

## /Heather Couch/

Heather Couch 22 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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<sup>&</sup>lt;sup>2</sup>The limited nucleotide sequencing of NR-22240 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.