

Certificate of Analysis for NR-22240

Human Metapneumovirus TN/93-32

Catalog No. NR-22240

Product Description: Cell lysate and supernatant from Macaca mulatta kidney epithelial cells¹ infected with human metapneumovirus (HMPV), TN/93-32

Passage History: LX/L6 (Vanderbilt/BEI Resources; L# = Number of passages in LLC-MK2 cells;

X = unknown)

Lot²: 62500403 Manufacturing Date: 24JUL2014

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in LLC-MK2 Derivative Cells ¹	Report results	Rounding, sloughing, and syncytia formation
Sequencing of Species-Specific Region ³ (F gene; 528 nucleotides)	Consistent with HMPV TN/93-32	99% identity with HMPV TN/93-32 (GenBank: KC562223)
Titer by TCID ₅₀ Assay ^{4,5} in LLC-MK2 Derivative Cells ¹ With Direct Fluorescence Assay (DFA) Readout ⁶	Report results	2.8 × 10 ⁵ TCID ₅₀ per mL
Sterility (21-day incubation)		
Harpo's HTYE broth ⁷ , 37°C and 26°C, aerobic	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 Test Article nucleic acid	None detected	None detected

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

Michael Q. Comba Signature: Date: 13 JAN 2015

> Title: Technical Manager, BEI Authentication or designee

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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²Grown in Opti-MEM® Minimal Essential Medium (Life Technologies 31985) supplemented with 2 mM L-glutamine (Media Tech 25-005-CI), 100 μg per mL CaCl₂, and 5 μg per mL trypsin (ATCC® 30-2101) for 6 days at 37°C and 5% CO₂

³The 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.

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 preparation.

⁵⁸ days at 37°C and 5% CO2

⁶Using Light Diagnostics™ Human Metapneumovirus DFA Reagent (Millipore 3124)

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