

Certificate of Analysis for NR-22234

Human Metapneumovirus, TN/91-320

Catalog No. NR-22234

Product Description: Cell lysate and supernatant from *Macaca mulatta* kidney epithelial cells¹ infected with human metapneumovirus (HMPV), TN/91-320

Passage History: L8/L7 (Vanderbilt/BEI Resources; L# = Number of passages in LLC-MK2 cells)

Lot²: 356 Manufacturing Date: 13FEB2017

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 (G and L genes; 1213 nucleotides)	Consistent with HMPV, TN/91-320	99% identity with HMPV, TN/91-320 (GenBank: KC403972)
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 ⁶ 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 extracted Test Article nucleic acid	None detected	None detected

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

Date: 26 JUL 2017

Signature:

BEI Resources Authentication

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 www.beiresources.org E-mail: contact@beiresources.org
Tel: 800-359-7370

Tel: 800-359-7370 Fax: 703-365-2898

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 6 days at 37°C and 5% CO₂

³The limited nucleotide sequencing of NR-22234 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.

 $^{^57}$ days at 37°C and 5% CO_2

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

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