

Certificate of Analysis for NR-22234

Human Metapneumovirus, TN/91-320

Catalog No. NR-22234

Product Description:

Human metapneumovirus (HMPV), TN/91-320 was isolated from a human specimen collected in Tennessee, USA, in 1991. NR-22234 lot 70044755 was produced by infecting *Macaca mulatta* kidney epithelial cells (LLC-MK2; ATCC[®] CCL-7.1™) with BEI Resources seed lot 62500401 and incubating in Opti-MEM[®] Minimal Essential Medium (Gibco; 31985-070) supplemented with 2 mM L-glutamine, 100 μg per mL CaCl₂, and 5 μg per mL trypsin for 7 days at 37°C with 5% CO₂.

Passage History:

L(8)/L(7) (Vanderbilt University Medical Center/BEI Resources); L = LLC-MK2

Lot: 70044755 Manufacturing Date: 02AUG2021

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TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in LLC-MK2 Derivative Cells	Syncytia formation and refractile rounding	Syncytia formation and refractile rounding
Sequencing of Species-Specific Region (~1220 nucleotides)	≥ 98% identity with HMPV, TN/91-320 (GenBank: KC403972)	99.5% identity with HMPV, TN/91-320 (GenBank: KC403972)
Titer by TCID ₅₀ Assay in LLC-MK2 Derivative Cells by Cytopathic Effect With Direct Fluorescence Assay (DFA) Readout ^{1,2} (8 days at 37°C and 5% CO ₂)	Report results	1.6 × 10 ⁶ TCID ₅₀ per mL
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic ³	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

¹The 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.
²Using Light Diagnostics™ Human Metapneumovirus DFA Reagent (Millipore 5091)

/Heather Couch/

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Program Manager or designee, ATCC Federal Solutions

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