b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-22227

Human Metapneumovirus, TN/83-1211

Catalog No. NR-22227

Product Description: Cell lysate and supernatant from *Macaca mulatta* kidney epithelial cells[1] infected with human metapneumovirus (HMPV), TN/83-1211

Passage History: L7/L3 (Vanderbilt/BEI Resources; L# = Number of passages in LLC-MK2 cells)

Lot[2]: 355 **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; 1221 nucleotides)	Consistent with HMPV, TN/83-1211	99% identity with HMPV, TN/83-1211 (GenBank: KC562244.1)
Titer by $TCID_{50}$ Assay[4,5] in LLC-MK2 Derivative Cells[1] With DFA Readout[6]	Report results	2.8×10^6 $TCID_{50}$ 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 Test Article nucleic acid	None detected	None detected

[1]LLC-MK2 Derivative cells (ATCC® CCL-7.1™)
[2]Grown in Opti-MEM® Minimal Essential Medium (Life Technologies 31985) supplemented with 2 mM L-glutamine (ATCC® 30-2214), 100 µg per mL $CaCl_2$ (Fisher BioReagents™ BP9742), and 5 µg per mL trypsin (ATCC® 30-2101) for 6 days at 37°C and 5% CO_2
[3]The limited nucleotide sequencing of NR-22227 performed at BEI Resources is not sufficient to confirm exact strain identity owing to the high degree of sequence conservation within HMPV lineages.
[4]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.
[5]7 days at 37°C and 5% CO_2
[6]Using Light Diagnostics™ Human Metapneumovirus DFA Reagent (Millipore 5091)
[7]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 19 JUN 2017 **Signature:** Michael R. Gyrla

BEI Resources Authentication

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