

Middle East Respiratory Syndrome Coronavirus, Isolate Hu/Jordan-N3/2012

Catalog No. NR-59312

Product Description:

Middle East Respiratory Syndrome (MERS) Coronavirus, Jordan-N3/2012, was isolated in April 2012 from a bronchial wash and sourced from the National Institutes of Health, with material deposited by the Integrated Research Facility, Fort Detrick, MD. NR-59312 lot 70059827 was produced by infecting Caco-2 cells (ATCC® HTB-37™) with MERS Coronavirus, Jordan-N3/2012 and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 2 days at 37°C with 5% CO₂. The cells and supernatant were spin-clarified at 1500 × g for 10 minutes at 4°C.

Total Passage: 2

Passage History:

V(1)/Ca(1) (IRF/BEI Resources); V = *Chlorocebus* (formerly *Cercopithecus*) *aethiops* kidney cells (Vero); Ca = Caco-2

Lot: 70059827

Manufacturing Date: 16FEB2023

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Caco-2 Cells	Syncytia formation and sloughing	Syncytia formation and sloughing
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with isolate Hu/Jordan-N3/2012 (GenBank: KC776174)	99.98% identity with isolate Hu/Jordan-N3/2012 (GenBank: KC776174)
Titer by TCID₅₀ Assay in Caco-2 Cells by Cytopathic Effect^{1,2} (5 days at 37°C and 5% CO ₂)	Report results	1.8 × 10 ⁸ TCID ₅₀ per mL
Endotoxin Content (<i>Limulus</i> Amoebocyte Lysate Assay)	Report results	≤ 0.03 EU per mL
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ³ Trypticase Soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Blood agar, 37°C, aerobic Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO ₂	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination Agar and broth culture (14-day incubation at 37°C) DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	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.

²Titer was determined by cytopathic effects (CPE) and completed in quadruplicate (1.6 × 10⁸ per mL, 2.8 × 10⁸ per mL, 2.8 × 10⁸ per mL, and 1.6 × 10⁷ per mL). The average of the four values is reported.

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

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15 FEB 2024

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APPENDIX I: NGS Information for NR-59312 lot 70059827

Sequence analysis using fastp 0.23.2 and variant caller LoFreq version: 2.1.5 resulted in the discovery of five SNPs when compared to Middle East Respiratory Syndrome virus, isolate Hu/Jordan-N3/2012 (GenBank: KC776174) (see Table I below).

Table I: Variants with different nucleotides between NR-59312 lot 70059827 and KC776174.1 (Human betacoronavirus 2c Jordan-N3/2012, complete genome)

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	T7276G	794	1	6.6751%	ORF1ab protein	Silent mutation
SNP	T19349A	1058	1	7.6560%	ORF1ab protein	L6373Q
SNP	T24045C	1563	1	34.6129%	S protein	I879T
SNP	T26948A	1245	1	8.1124%	ORF5 protein	L52Q
SNP	T27490C	1578	1	7.6046%	Intergenic	Untranslated