

Middle East Respiratory Syndrome-Related Coronavirus, Isolate Hu/Riyadh-KSA-18013832/2018

Catalog No. NR-59606

Product Description:

Middle East Respiratory Syndrome-Related coronavirus (MERS-CoV), isolate Hu/Riyadh-KSA-18013832/2018 was isolated from a nasopharyngeal swab from a patient with respiratory illness on August 30, 2018, in the Kingdom of Saudi Arabia (KSA). NR-59606 lot 70070749 was produced by infecting human colorectal adenocarcinoma cells (Caco-2; ATCC® HTB-37™) with the deposited material 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 to produce this lot. The cells and supernatant were spin-clarified at 1500 × g for 10 minutes at 4°C.

Passage History:

V(3)/C(1) (Centers for Disease Control and Prevention/BEI Resources); V = Vero cells, C = Caco-2

Lot: 70070749

Manufacturing Date: 16AUG2024

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Caco-2 Cells	Cell rounding and syncytia formation	Cell rounding and syncytia formation
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with MERS, Hu/Riyadh-KSA-18013832/2018 (GenBank: MN723544)	99.98% identity with MERS, Hu/Riyadh-KSA-18013832/2018 (GenBank: MN723544)
Genome Copy (GC) Number by qPCR	Report results	9.4 × 10 ⁹ GC per mL
Titer by TCID₅₀ Assay in Caco-2 Cells by Cytopathic Effect^{1,2} (5 days at 37°C with 5% CO ₂)	Report results	1.4 × 10 ⁸ TCID ₅₀ /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 Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C, aerobic	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 effect (CPE) and completed in triplicate (8.9 × 10⁷ per mL, 1.6 × 10⁸ per mL and 1.6 × 10⁸ per mL). The average of the three values is reported.

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

/Sonia Bjorum Brower/

Sonia Bjorum Brower

13 SEP 2024

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APPENDIX I: NGS Information for NR-59606 lot 70070749

Sequence analysis using fastp 0.23.4 and variant caller LoFreq resulted in the discovery of seven SNPs when compared to Middle East respiratory syndrome-related coronavirus strain Hu/Riyadh-KSA-18013832/2018, complete genome (GenBank: MK462253.1) (see Table I below).

Table I: Variants with different nucleotides between NR-59606 lot 70070749 and MK462253.1 (Middle East respiratory syndrome-related coronavirus strain Hu/Riyadh-KSA-18013832/2018, complete genome)

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	C32T	491	1	100.0000%	3'UTR	Untranslated
SNP	A15629G	512	1	5.6641%	ORF1ab	K5118E
SNP	T19395A	662	1	8.1571%	ORF1ab	L6373Q
SNP	C21149A	423	1	100.0000%	ORF1ab	L6958I
SNP	C25009T	1236	1	100.0000%	S protein	S1185L
SNP	T26994A	707	1	5.9406%	ORF5	L52Q
SNP	T26994G	707	1	6.0820%	ORF5	L52Q