

SARS-Related Coronavirus 2, Isolate Singapore/2/2020

Catalog No. NR-52369

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate Singapore/2/2020 was isolated in January 2020 from a throat swab from a human patient in Singapore. NR-52369 lot 70035611 was produced by infecting *Cercopithecus aethiops* kidney cells (Vero E6; ATCC® CRL-1586™) with the deposited material 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.

Passage History:

VE6(4)/VE6(2) (Duke-National University of Singapore Medical School/BEI Resources); VE6 = Vero E6 cells

Lot: 70035611

Manufacturing Date: 21MAY2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 Cells	Cell rounding and detachment	Cell rounding and detachment
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with SARS-CoV-2	≥ 98% identity with SARS-CoV-2 ¹
Titer by TCID₅₀ Assay in Vero E6 Cells by Cytopathic Effect² (6 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 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 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

¹Sequence information for SARS-CoV-2, Singapore/02/2020 is not available in the NCBI database; nucleotide sequence obtained for NR-52369 lot 70035611 is 99.97% identical to SARS-CoV-2 isolate Wuhan-Hu-1, complete genome (GenBank: MN908947.3) and consistent with numerous SARS-CoV-2 strains.

²The 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.

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

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APPENDIX I: NGS Information for NR-52369 lot 70035611

Sequence analysis resulted in the discovery of five SNPs and one deletion when compared to the reference sequence from GISAID EPI_ISL_407987 (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52369_70035611 Sequence	Position in EPI_ISL_407987 Reference Sequence	Reported EPI_ISL_407987 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
507	516	TATG	T	n/a	Indel	3	0.5660127
22084	22096	C	T	104	SNP	1	0.2127660
22085	22097	T	C	108	SNP	1	0.2145923
22087	22099	G	A	104	SNP	1	0.2127660
22283	22295	A	G	186	SNP	1	0.2827869
23603	23615	G	A	228	SNP	1	0.9036697