

SARS-Related Coronavirus 2, Isolate hCoV-19/Japan/TY7-503/2021 (Brazil P.1)

Catalog No. NR-54982

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/Japan/TY7-503/2021 (Brazil P.1) was isolated in airport quarantine in Japan from a COVID-19 positive passenger from Brazil in January 2021. NR-54982 lot 70042875 was produced by infecting *Homo sapiens* lung adenocarcinoma cells (Calu-3; ATCC® HTB-55™) with the deposited material and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) and 0.1% penicillin/streptomycin solution for 2 days at 37°C with 5% CO₂. The cells and supernatant were spin-clarified at 1000 × g for 10 minutes at 4°C.

Passage History:

VE6-TMPRSS2(2)/C(1) (National Institute of Infectious Diseases/BEI Resources); VE6-TMPRSS2 = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene; C = Calu-3 cells

Lot: 70042875

Manufacturing Date: 05MAR2021

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TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Calu-3 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, hCoV-19/Japan/IC-0564/2021 (GISAID: EPI_ISL_792683)	100% identity with SARS-CoV-2, hCoV-19/Japan/IC-0564/2021 (GISAID: EPI_ISL_792683)
Titer by TCID₅₀ Assay in Calu-3 Cells by Cytopathic Effect¹ (5 days at 37°C and 5% CO ₂)	Report results	2.8 × 10 ⁶ TCID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ² 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.

²Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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13 APR 2021

Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-54982 lot 70042875

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of two SNPs when compared to the reference sequence from GISAID EPI_ISL_792683 (see Table I below). Additionally, both the reference sequence EPI_ISL_792683 and NR-54982 lot 70042875 contained thirty-four SNPs, one insertion and one deletion (Indel) when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Table I: Variants with different nucleotides between NR-54982 lot 70042875 and reference sequence EPI_ISL_792683

Position in NR-54982_70042875 Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Position in EPI_ISL_792683 Reference Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_792683 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant (%)
11483	11522	11483	T	T	G	49314	SNP	1	94.1378
14640	14679	14640	T	T	C	1296	SNP	1	7.3363

Table II: Variants with different nucleotides between NR-54982 lot 70042875 and GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Position in NR-54982_70042875 Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Position in EPI_ISL_792683 Reference Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_792683 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant (%)
211	241	211	C	T	T	49314	SNP	1	99.7390
703	733	703	T	C	C	49314	SNP	1	99.9342
2719	2749	2719	C	T	T	49314	SNP	1	100.0000
3007	3037	3007	C	T	T	49314	SNP	1	99.7933
3798	3828	3798	C	T	T	49314	SNP	1	99.5482
5618	5648	5618	A	C	C	49314	SNP	1	99.7252
6289	6319	6289	A	G	G	49314	SNP	1	99.9790
6583	6613	6583	A	G	G	49314	SNP	1	99.9018
11257	11287	11257	GTCTGGT TTTA	G-----A	G-----A	70163	Indel	-9	100.0000
12739	12778	12739	C	T	T	49314	SNP	1	99.8914
13821	13860	13821	C	T	T	49314	SNP	1	99.8885
14369	14408	14369	C	T	T	49314	SNP	1	98.2968
17220	17259	17220	G	T	T	49314	SNP	1	100.0000
21575	21614	21575	C	T	T	49314	SNP	1	99.8656
21582	21621	21582	C	A	A	49314	SNP	1	99.9208
21599	21638	21599	C	T	T	49314	SNP	1	99.6204
21935	21974	21935	G	T	T	49314	SNP	1	99.6043
22093	22132	22093	G	T	T	49314	SNP	1	99.9571
22773	22812	22773	A	C	C	49314	SNP	1	99.9564
22973	23012	22973	G	A	A	49314	SNP	1	99.9054
23024	23063	23024	A	T	T	49314	SNP	1	99.9062
23364	23403	23364	A	G	G	49314	SNP	1	99.9002
23486	23525	23486	C	T	T	49314	SNP	1	99.9374
24603	24642	24603	C	T	T	49314	SNP	1	99.9076
25049	25088	25049	G	T	T	49314	SNP	1	99.9610
26110	26149	26110	T	C	C	49314	SNP	1	99.9396
28128	28167	28128	G	A	A	49314	SNP	1	99.0810

Position in NR-54982_70042875 Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Position in EPI_ISL_792683 Reference Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_792683 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant (%)
28223	28262	28223	G----- AACAAAC T	GAACAAAC AAACT	GAACAAA CAAAC	129008	Indel	+4	99.9913
28477	28512	28477	C	G	G	49314	SNP	1	99.9404
28842	28877	28842	A	T	T	49314	SNP	1	98.2949
28843	28878	28843	G	C	C	49314	SNP	1	99.3187
28846	28881	28846	G	A	A	49314	SNP	1	99.3358
28847	28882	28847	G	A	A	49314	SNP	1	99.3183
28849	28883	28848	G	C	C	49314	SNP	1	99.4093
29800	29834	N/A	T	N/A	A	49314	SNP	1	99.8731
29824	29858	N/A	T	N/A	A	43982	SNP	1	99.8450