# Certificate of Analysis for NR-56225

## SARS-Related Coronavirus 2, Isolate hCoV-19/USA/MD-HP06587/2021 (Lineage B.1.621.1; Mu Variant)

## Catalog No. NR-56225

## Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/MD-HP06587/2021 was isolated from a human on June 15, 2021, in Maryland, USA. NR-56225 lot 70048750 was produced by infecting *Homo sapiens* lung adenocarcinoma epithelial 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™) for 3 days at 37°C with 5% $CO_2$. The cells and supernatant were spin-clarified at 1500 × g for 10 minutes at 4°C.

## Passage History:

V(1)/C(1) (Johns Hopkins University/BEI Resources); V = *Cercopithecus aethiops* kidney cells; C = Calu-3

## Lot: 70048750 Manufacturing Date: 08NOV2021

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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 isolate hCoV-19/USA/MD-HP06587/2021 (GISAID: EPI_ISL_3243079) | 99.99% identity with isolate hCoV-19/USA/MD-HP06587/2021 (GISAID: EPI_ISL_3243079) |
| **Titer by $TCID_{50}$ Assay in Calu-3 Cells by Cytopathic Effect**[1] (7 days at 37°C and 5% $CO_2$) | Report results | $2.4 \times 10^6$ $TCID_{50}$ per mL[2] |
| **Sterility (21-day incubation)** | | |
| Harpo's HTYE broth, 37°C and 26°C, aerobic[3] | 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, aerobic | 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 extracted Test Article nucleic acid | None detected | None detected |

[1]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.
[2]Titer was determined by cytopathic effects (CPE) and completed in triplicate ($1.6 \times 10^6$ per mL, $2.8 \times 10^6$ per mL and $2.8 \times 10^6$ per mL). The average of the three values is reported.
[3]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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/Sonia Bjorum Brower/
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Technical Manager or designee, ATCC Federal Solutions

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## APPENDIX I: NGS Information for NR-56225 lot 70048750

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant callers LoFreq version: 2.1.5 and freebayes version: v1.3.1-dirty resulted in the discovery of one SNP when compared to the reference sequence from GISAID EPI_ISL_3243079 (see Table I below). Additionally, both the reference sequence EPI_ISL_3243079 and NR-56225 lot 70048750 contained thirty-three SNPs, one insertion (INS) and one deletion (DEL) 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-56225 lot 70048750 and reference sequence EPI_ISL_3243079**

| Variant Type | Variant Position and Identified Alternative Base | Coverage | Length of Variant | Frequency of Variant | Gene (Region) | Amino Acid Mutation |
|---|---|---|---|---|---|---|
| SNP | t14679c | 1583 | 1 | 6.0013% | ORF1ab (nsp12) | Silent mutation |

**Table II: Variants with different nucleotides between NR-56225 lot 70048750 and GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)**

| Variant Type | Variant Position and Identified Alternative Base | Coverage[1] | Length of Variant | Frequency of Variant[1] | Gene (Region) | Amino Acid Mutation |
|---|---|---|---|---|---|---|
| SNP | c241t | N/A | 1 | 100.0000% | 5'UTR | Untranslated |
| SNP | a1818g | N/A | 1 | 100.0000% | ORF1ab (nsp2) | K338R |
| SNP | c3037t | N/A | 1 | 100.0000% | ORF1ab (nsp3) | Silent mutation |
| SNP | a3428g | N/A | 1 | 100.0000% | ORF1ab (nsp3) | T237A |
| SNP | c4878t | N/A | 1 | 100.0000% | ORF1ab (nsp3) | T720I |
| SNP | c5192t | N/A | 1 | 100.0000% | ORF1ab (nsp3) | Silent mutation |
| SNP | c6037t | N/A | 1 | 100.0000% | ORF1ab (nsp3) | Silent mutation |
| SNP | t6842g | N/A | 1 | 100.0000% | ORF1ab (nsp3) | S1375A |
| SNP | c10029t | N/A | 1 | 100.0000% | ORF1ab (nsp4) | T492I |
| SNP | a11451g | N/A | 1 | 100.0000% | ORF1ab (nsp6) | Q160R |
| SNP | a13057t | N/A | 1 | 100.0000% | ORF1ab (nsp10) | Silent mutation |
| SNP | c14408t | N/A | 1 | 100.0000% | ORF1ab (nsp12) | P323L |
| SNP | c17491t | N/A | 1 | 100.0000% | ORF1ab (nsp13) | P419S |
| SNP | c17707t | N/A | 1 | 100.0000% | ORF1ab (nsp13) | P491S |
| SNP | c18877t | N/A | -9 | 100.0000% | ORF1ab (nsp14) | Silent mutation |
| SNP | t19035c | N/A | 1 | 100.0000% | ORF1ab (nsp14) | Silent mutation |
| SNP | c20148t | N/A | 1 | 100.0000% | ORF1ab (nsp15) | Silent mutation |

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| Variant Type | Variant Position and Identified Alternative Base | Coverage[1] | Length of Variant | Frequency of Variant[1] | Gene (Region) | Amino Acid Mutation |
|---|---|---|---|---|---|---|
| SNP | c21846t | N/A | 1 | 100.0000% | Spike | T95I |
| INS | 21990[tac]21991 | N/A | +3 | 100.0000% | Spike | V143[T]Y144 |
| SNP | t21995a | N/A | 1 | 100.0000% | Spike | Y145N |
| SNP | g22599a | N/A | 1 | 100.0000% | Spike | R346K |
| SNP | g23012a | N/A | 1 | 100.0000% | Spike | E484K |
| SNP | a23063t | N/A | 1 | 100.0000% | Spike | N501Y |
| SNP | a23403g | N/A | 1 | 100.0000% | Spike | D614G |
| SNP | c23604a | N/A | 1 | 100.0000% | Spike | P681H |
| SNP | g24410a | N/A | 1 | 100.0000% | Spike | D950N |
| SNP | g25563t | N/A | 1 | 100.0000% | ORF3a | Q57H |
| DEL | Δ26158-26161 | N/A | -4 | 100.0000% | ORF3a | V256I, N257Q, Δamino acids 258-275 |
| SNP | a26492t | N/A | 1 | 100.0000% | Intergenic - Envelope/Membrane | Untranslated |
| SNP | c27925a | N/A | 1 | 100.0000% | ORF8 | T11K |
| SNP | c28005t | N/A | 1 | 100.0000% | ORF8 | P38S |
| SNP | c28093t | N/A | 1 | 100.0000% | ORF8 | S67F |
| SNP | a28272t | N/A | 1 | 100.0000% | Intergenic - ORF8/Nucleocapsid | Untranslated |
| SNP | c28887t | N/A | 1 | 100.0000% | Nucleocapsid | T250I |
| SNP | g29779t | N/A | 1 | 100.0000% | 3'UTR | Untranslated |

[1]Coverage for all variants in Table II is listed as 'N/A'. There is no read coverage information for these variants because the sample reads are only mapped to the reference sequence and not to the SARS-CoV-2, Wuhan-Hu-1 isolate sequence (GenBank MN908947), but that does not mean these areas lack for coverage. All variants in Table II are mismatches in between the reference sequence and the SARS-CoV-2, Wuhan-Hu-1 sequence, so these variants will be assigned a frequency of 100% and will therefore be majority SNPs.

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