

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/PHC248/2021 (Lineage B.1.526; Iota Variant)

Catalog No. NR-55609

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/PHC248/2021 was isolated from a human nasal swab on April 20, 2020, in Memphis, Tennessee, USA. NR-55609 lot 70048991 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% CO₂. The cells and supernatant were spin-clarified at 1500 × g for 10 minutes at 4°C.

Passage History:

VTA(1)VT(1)/C(1) (St. Jude Children's Research Hospital/BEI Resources); VTA = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene and human ACE2; VT = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene; C = Calu-3

Lot: 70048991

Manufacturing Date: 19NOV2021

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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/PHC248/2021 (GenBank: OL442160) | 99.99% identity with isolate hCoV-19/USA/PHC248/2021 (GenBank: OL442160) |
| Titer by TCID₅₀ Assay in Calu-3 Cells by Cytopathic Effect¹ (5 days at 37°C and 5% CO ₂) | Report results | 2.4 × 10 ⁶ TCID ₅₀ per mL ² |
| Endotoxin Content (<i>Limulus</i> Amoebocyte Lysate Assay) | Report results | ≤ 0.04 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 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 effects (CPE) and completed in triplicate (1.6 × 10⁶ per mL, 2.8 × 10⁶ per mL and 2.8 × 10⁶ 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.

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APPENDIX I: NGS Information for NR-55609 lot 70048991

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 two SNPs when compared to the reference sequence GenBank OL442160.1 (see Table I below). Additionally, both the reference sequence GenBank OL442160.1 and NR-55609 lot 70048991 contained twenty-eight SNPs and two DEL when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below). At nucleotide 25, there is a reversion of the original mutation “t” in reference genome OL442160.1 back to a “c” as found in GenBank MN908947 (see Table III below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Table I: Variants with different nucleotides between NR-55609 lot 70048991 and GenBank OL442160.1

| Variant Type | Variant Position and Identified Alternative Base | Coverage | Length of Variant | Frequency of Variant | Gene (Region) | Amino Acid Mutation |
|--------------|--|----------|-------------------|----------------------|---------------|---------------------|
| SNP | c11750t | 3282 | 1 | 59.0494% | ORF1ab (nsp6) | L260F |
| SNP | a29871g | 149 | 1 | 34.8993% | 3'UTR | Untranslated |

Table II: Variants with different nucleotides between NR-55609 lot 70048991 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 | c1059t | N/A | 1 | 100.0000% | ORF1ab (nsp2) | T85I |
| SNP | c1102t | N/A | 1 | 100.0000% | ORF1ab (nsp2) | Silent mutation |
| SNP | c3037t | N/A | 1 | 100.0000% | ORF1ab (nsp3) | Silent mutation |
| SNP | g6101a | N/A | 1 | 100.0000% | ORF1ab (nsp3) | G1128S |
| SNP | a7201g | N/A | 1 | 100.0000% | ORF1ab (nsp3) | Silent mutation |
| SNP | t9867c | N/A | 1 | 100.0000% | ORF1ab (nsp4) | L438P |
| SNP | c9943t | N/A | 1 | 100.0000% | ORF1ab (nsp4) | Silent mutation |
| SNP | c10450t | N/A | 1 | 100.0000% | ORF1ab (nsp5) | Silent mutation |
| DEL | Δ11288-11296 | N/A | -9 | 100.0000% | ORF1ab (nsp6) | ΔSGF (amino acids 106-108) |
| SNP | c14408t | N/A | 1 | 100.0000% | ORF1ab (nsp12) | P323L |
| SNP | c14649t | N/A | 1 | 100.0000% | ORF1ab (nsp12) | Silent mutation |
| SNP | a16500c | N/A | 1 | 100.0000% | ORF1ab (nsp13) | Q88H |
| SNP | c16733t | N/A | 1 | 100.0000% | ORF1ab (nsp13) | S166L |
| SNP | t16794c | N/A | 1 | 100.0000% | ORF1ab (nsp13) | Silent mutation |
| SNP | a20262g | N/A | 1 | 100.0000% | ORF1ab (nsp15) | Silent mutation |

| Variant Type | Variant Position and Identified Alternative Base | Coverage ¹ | Length of Variant | Frequency of Variant ¹ | Gene (Region) | Amino Acid Mutation |
|--------------|--|-----------------------|-------------------|-----------------------------------|---------------------|---------------------|
| SNP | c21058t | N/A | 1 | 100.0000% | ORF1ab (nsp16) | P134S |
| SNP | c21575t | N/A | 1 | 100.0000% | Spike | L5F |
| SNP | c21846t | N/A | 1 | 100.0000% | Spike | T95I |
| SNP | a22320g | N/A | 1 | 100.0000% | Spike | D253G |
| SNP | a22554g | N/A | 1 | 100.0000% | Spike | N331S |
| SNP | a23403g | N/A | 1 | 100.0000% | Spike | D614G |
| SNP | c23664t | N/A | 1 | 100.0000% | Spike | A701V |
| SNP | c25517t | N/A | 1 | 100.0000% | ORF3a | P42L |
| SNP | g25563t | N/A | 1 | 100.0000% | ORF3a | Q57H |
| SNP | c27925t | N/A | 1 | 100.0000% | ORF8 | T11I |
| SNP | g28077t | N/A | 1 | 100.0000% | ORF8 | V62L |
| DEL | Δ28271 | N/A | -1 | 100.0000% | Intergenic (ORF8/N) | Untranslated |
| SNP | c28869t | N/A | 1 | 100.0000% | Nucleocapsid | P199L |
| SNP | g28975a | N/A | 1 | 100.0000% | Nucleocapsid | M234I |

¹Coverage 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.

Table III: Variants with different nucleotides between NR-55609 lot 70048991 and MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) compared with GenBank OL442160.1

| Variant Type | Variant Position and Identified Alternative Base | Coverage | Length of Variant | Frequency of Variant | Gene (Region) | Amino Acid Mutation |
|--------------|--|----------|-------------------|----------------------|---------------|---------------------|
| SNP | t25c_rev_t ² | 263 | 1 | 100.0000% | 5'UTR | Untranslated |

²rev = reversion. t25c represents the original mutation from "t" in Wuhan-Hu-1 to "c" in the provided reference genome OL442160.1. _rev_t represents the reversion back to t in NR-55609 lot 70048991.