

SARS-Related Coronavirus 2, Isolate hCoV-19/USA-NJ-CVD124/2020 (Lineage R.1)

Catalog No. NR-55469

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA-NJ-CVD124/2020 was isolated from a nasopharyngeal swab in New Jersey, USA in November 2020. NR-55469 lot 70044692 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™) for 5 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:

VT2(1)/C(1) (Center for Discovery and Innovation/BEI Resources); VT2 = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 (TMPRSS2) gene; C = Calu-3 cells

Lot: 70044692

Manufacturing Date: 16MAY2021

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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/USA/NJ-NYGC-CVD-124-Quest8/2020 (GISAID: EPI_ISL_2008060) | 99.97% identity with SARS-CoV-2, hCoV-19/USA/NJ-NYGC-CVD-124-Quest8/2020 (GISAID: EPI_ISL_2008060) |
| Titer by TCID ₅₀ Assay in Calu-3 Cells by Cytopathic Effect ¹ (5 days at 37°C and 5% CO ₂) | Report results | 1.3 × 10 ⁴ TCID ₅₀ per mL ² |
| 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 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 (2.8 × 10³ per mL, 2.8 × 10⁴ per mL and 8.9 × 10³ 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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Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-55469 lot 70044692

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of ten SNPs when compared to the reference sequence from GISAID EPI_ISL_2008060 (see Table I below). Additionally, both the reference sequence EPI_ISL_2008060 and NR-55469 lot 70044692 contained twenty-three SNPs and one deletion (DEL) when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below).

Table I: Variants with different nucleotides between NR-55469 lot 70044692 and reference sequence EPI_ISL_2008060

| Variant Type | Variant Position and Identified Alternative Base | Coverage | Length of Variant | Frequency of Variant | Gene (Region) | Amino Acid Mutation |
|--------------|--|----------|-------------------|----------------------|------------------|---------------------|
| SNP | a4291g | 193 | 1 | 5.1813% | ORF1ab (nsp3) | Silent mutation |
| SNP | c7749t | 70 | 1 | 11.4286% | ORF1ab (nsp3) | T1677I |
| SNP | c12115t | 150 | 1 | 51.3333% | ORF1ab (nsp8) | Silent mutation |
| SNP | t14679c | 86 | 1 | 12.7907% | ORF1ab (nsp12) | Silent mutation |
| SNP | c19983t | 287 | 1 | 51.2195% | ORF1ab (nsp15) | Silent mutation |
| SNP | a22678g | 106 | 1 | 6.6038% | Spike | Silent mutation |
| SNP | t26336a | 145 | 1 | 8.2759% | Envelope protein | L31Q |
| SNP | c26681t | 161 | 1 | 8.0745% | Membrane protein | Silent mutation |
| SNP | c26728t | 155 | 1 | 45.1613% | Membrane protein | A69V |
| SNP | t26791g | 176 | 1 | 9.0909% | Membrane protein | L90W |

Table II: Variants with different nucleotides between NR-55469 lot 70044692 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 region |
| SNP | c3037t | N/A | 1 | 100.0000% | ORF1ab (nsp3) | Silent mutation |
| SNP | t7685g | N/A | 1 | 100.0000% | ORF1ab (nsp3) | S1656A |
| SNP | c14340t | N/A | 1 | 100.0000% | ORF1ab (nsp12) | Silent mutation |
| SNP | c14408t | N/A | 1 | 100.0000% | ORF1ab (nsp12) | P323L |
| SNP | a16569g | N/A | 1 | 100.0000% | ORF1ab (nsp13) | Silent mutation |
| SNP | g17551a | N/A | 1 | 100.0000% | ORF1ab (nsp13) | G439R |
| SNP | c18877t | N/A | 1 | 100.0000% | ORF1ab (nsp14) | Silent mutation |
| SNP | a19167g | N/A | 1 | 100.0000% | ORF1ab (nsp14) | 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 | c19274a | N/A | 1 | 100.0000% | ORF1ab (nsp14) | P412H |
| SNP | t19839c | N/A | 1 | 100.0000% | ORF1ab (nsp15) | Silent mutation |
| SNP | g22017t | N/A | 1 | 100.0000% | Spike | W152L |
| SNP | c22326t | N/A | 1 | 100.0000% | Spike | S255F |
| SNP | g23012a | N/A | 1 | 100.0000% | Spike | E484K |
| SNP | a23403g | N/A | 1 | 100.0000% | Spike | D614G |
| SNP | g23868t | N/A | 1 | 100.0000% | Spike | G769V |
| SNP | t26604c | N/A | 1 | 100.0000% | Membrane protein | F28L |
| SNP | c27213t | N/A | 1 | 100.0000% | ORF6 | Silent mutation |
| DEL | Δ28271 | N/A | -1 | 100.0000% | Intergenic (ORF8/Nucleocapsid) | Untranslated region |
| SNP | c28833t | N/A | 1 | 100.0000% | Nucleocapsid protein | S187L |
| SNP | g28881a | N/A | 1 | 100.0000% | Nucleocapsid protein | R203K |
| SNP | g28882a | N/A | 1 | 100.0000% | | |
| SNP | g28883c | N/A | 1 | 100.0000% | Nucleocapsid protein | G204R |
| SNP | g29527t | N/A | 1 | 100.0000% | Nucleocapsid protein | Q418H |

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.