# Certificate of Analysis for NR-59204

## SARS-Related Coronavirus 2, Isolate hCoV-19/USA/MD-HP41275/2022 (Lineage CH.1.1; Omicron Variant)

## Catalog No. NR-59204

## Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/MD-HP41275/2022 was isolated from a human on November 25, 2022, in Maryland, USA. NR-59204 lot 70058693 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells expressing transmembrane protease, serine 2 and human angiotensin-converting enzyme 2 (Vero E6-TMPRSS2-T2A-ACE2; VTA; BEI Resources NR-54970) with the deposited material and incubating with Eagle's Minimum Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate and 1.5 g/L of sodium bicarbonate (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:

VT(1)/VTA(1) (Johns Hopkins University/BEI Resources); VT = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene (Vero E6-TMPRSS2); VTA = *Cercopithecus aethiops* kidney cells expressing transmembrane protease, serine 2 gene and human angiotensin-converting enzyme 2 (Vero E6-TMPRSS2-T2A-ACE2)

## Lot: 70058693 Manufacturing Date: 13FEB2023

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Identification by Infectivity in VTA 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-HP41275/2022 (GISAID: EPI_ISL_16253902) | 99.97% identity with isolate hCoV-19/USA/MD-HP41275/2022 (GISAID: EPI_ISL_16253902) |
| **Titer by $TCID_{50}$ Assay in VTA Cells by Cytopathic Effect**[1,2] (7 days at 37°C and 5% $CO_2$) | Report results | $7.9 \times 10^5$ $TCID_{50}$ per mL |
| **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 |
| Blood agar, 37°C, aerobic | No growth | No growth |
| Blood agar, 37°C, anaerobic | No growth | No growth |
| Thioglycollate broth, 37°C, anaerobic | No growth | No growth |
| DMEM with 10% FBS, 37°C and 5% $CO_2$ | 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 ($2.8 \times 10^5$ per mL, $1.6 \times 10^6$ per mL and $5.0 \times 10^5$ 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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Technical Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.



Support Provided by NIAID

## APPENDIX I: NGS Information for NR-59204 lot 70058693

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant caller LoFreq version: 2.1.5 resulted in the discovery of eight SNPs when compared to GISAID EPI_ISL_16253902 (see Table I below). Additionally, both the modified reference sequence EPI_ISL_16253902 and NR-59204 lot 70058693 contained ninety SNPs and five 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-59204 lot 70058693 and modified reference sequence EPI_ISL_16253902**

| Variant Type | Variant Position and Identified Alternative Base | Coverage | Length of Variant | Frequency of Variant | Gene (Region) | Amino Acid Mutation |
|---|---|---|---|---|---|---|
| SNP | a8821g | 560 | 1 | 5.3571% | ORF1ab (nsp4) | Silent mutation |
| SNP | c11454t | 1015 | 1 | 45.0246% | ORF1ab (nsp6) | A161V |
| SNP | c11750t | 1494 | 1 | 15.2610% | ORF1ab (nsp6) | L260F |
| SNP | c12357t | 1073 | 1 | 28.3318% | ORF1ab (nsp8) | T89I |
| SNP | a13131g | 651 | 1 | 5.0691% | ORF1ab (nsp10) | Q36R |
| SNP | t14679c | 545 | 1 | 12.1101% | ORF1ab (nsp12) | Silent mutation |
| SNP | t15357c | 768 | 1 | 8.3333% | ORF1ab (nsp12) | Silent mutation |
| SNP | a25806g | 576 | 1 | 6.5972 % | ORF3a | Silent mutation |

**Table II: Variants with different nucleotides between NR-59204 lot 70058693 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 | t670g | N/A | 1 | 100.0000% | ORF1ab (nsp1) | S135R |
| SNP | c697t | N/A | 1 | 100.0000% | ORF1ab (nsp1) | Silent mutation |
| SNP | g1397a | N/A | 1 | 100.0000% | ORF1ab (nsp2) | V198I |
| SNP | c2790t | N/A | 1 | 100.0000% | ORF1ab (nsp3) | T24I |
| SNP | c3037t | N/A | 1 | 100.0000% | ORF1ab (nsp3) | Silent mutation |
| SNP | c3796t | N/A | 1 | 100.0000% | ORF1ab (nsp3) | Silent mutation |
| SNP | c3857a | N/A | 1 | 100.0000% | ORF1ab (nsp3) | Q380K |
| SNP | c3927t | N/A | 1 | 100.0000% | ORF1ab (nsp3) | S403L |
| SNP | g4184a | N/A | 1 | 100.0000% | ORF1ab (nsp3) | G489S |
| SNP | c4321t | N/A | 1 | 100.0000% | ORF1ab (nsp3) | 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 | c4586t | N/A | 1 | 100.0000% | ORF1ab (nsp3) | Silent mutation |
| SNP | c5183t | N/A | 1 | 100.0000% | ORF1ab (nsp3) | P822S |
| SNP | c9344t | N/A | 1 | 100.0000% | ORF1ab (nsp4) | L264F |
| SNP | a9424g | N/A | 1 | 100.0000% | ORF1ab (nsp4) | Silent mutation |
| SNP | c9534t | N/A | 1 | 100.0000% | ORF1ab (nsp4) | T327I |
| SNP | c9866t | N/A | 1 | 100.0000% | ORF1ab (nsp4) | L438F |
| SNP | c10029t | N/A | 1 | 100.0000% | ORF1ab (nsp4) | T492I |
| SNP | c10198t | N/A | 1 | 100.0000% | ORF1ab (nsp5) | Silent mutation |
| SNP | g10447a | N/A | 1 | 100.0000% | ORF1ab (nsp5) | Silent mutation |
| SNP | c10449a | N/A | 1 | 100.0000% | ORF1ab (nsp5) | P132H |
| DEL | Δ11288-11296 | N/A | -9 | 100.0000% | ORF1ab (nsp6) | ΔSGF (amino acids 106-108) |
| SNP | a12444g | N/A | 1 | 100.0000% | ORF1ab (nsp8) | N118S |
| SNP | c12880t | N/A | 1 | 100.0000% | ORF1ab (nsp9) | Silent mutation |
| SNP | c14408t | N/A | 1 | 100.0000% | ORF1ab (nsp12) | P323L |
| SNP | g15451a | N/A | 1 | 100.0000% | ORF1ab (nsp12) | G671S |
| SNP | c15714t | N/A | 1 | 100.0000% | ORF1ab (nsp12) | Silent mutation |
| SNP | c17410t | N/A | 1 | 100.0000% | ORF1ab (nsp13) | R392C |
| SNP | a18163g | N/A | 1 | 100.0000% | ORF1ab (nsp14) | I42V |
| SNP | g18583a | N/A | 1 | 100.0000% | ORF1ab (nsp14) | V182I |
| SNP | c19955t | N/A | 1 | 100.0000% | ORF1ab (nsp15) | T112I |
| SNP | a20055g | N/A | 1 | 100.0000% | ORF1ab (nsp15) | Silent mutation |
| SNP | a20741g | N/A | 1 | 100.0000% | ORF1ab (nsp16) | Q28R |
| SNP | c21618t | N/A | 1 | 100.0000% | Spike | T19I |
| DEL | Δ21633-21641 | N/A | -9 | 100.0000% | Spike | A27S ΔLPP (amino acids 24-26) |
| SNP | c21811t | N/A | 1 | 100.0000% | Spike | Silent mutation |
| SNP | g21987a | N/A | 1 | 100.0000% | Spike | G142D |
| SNP | a22001g | N/A | 1 | 100.0000% | Spike | K147E |
| SNP | t22016c | N/A | 1 | 100.0000% | Spike | W152R |
| SNP | c22033a | N/A | 1 | 100.0000% | Spike | F157L |
| SNP | a22190g | N/A | 1 | 100.0000% | Spike | I210V |
| SNP | t22200a | N/A | 1 | 100.0000% | Spike | V213G |
| SNP | g22331a | N/A | 1 | 100.0000% | Spike | G257S |
| SNP | g22577c | N/A | 1 | 100.0000% | Spike | G339H |
| SNP | g22578a | N/A | 1 | 100.0000% | | |
| SNP | g22599c | N/A | 1 | 100.0000% | Spike | R346T |
| SNP | c22674t | N/A | 1 | 100.0000% | Spike | S371F |
| SNP | t22679c | N/A | 1 | 100.0000% | Spike | S373P |
| SNP | c22686t | N/A | 1 | 100.0000% | Spike | S375F |

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|---|---|---|---|---|---|---|
| SNP | a22688g | N/A | 1 | 100.0000% | Spike | T376A |
| SNP | g22775a | N/A | 1 | 100.0000% | Spike | D405N |
| SNP | a22786c | N/A | 1 | 100.0000% | Spike | R408S |
| SNP | g22813t | N/A | 1 | 100.0000% | Spike | K417N |
| SNP | t22882g | N/A | 1 | 100.0000% | Spike | N440K |
| SNP | a22893c | N/A | 1 | 100.0000% | Spike | K444T |
| SNP | g22898a | N/A | 1 | 100.0000% | Spike | G446S |
| SNP | t22917g | N/A | 1 | 100.0000% | Spike | L452R |
| SNP | t22942g | N/A | 1 | 100.0000% | Spike | N460K |
| SNP | g22992a | N/A | 1 | 100.0000% | Spike | S477N |
| SNP | c22995a | N/A | 1 | 100.0000% | Spike | T478K |
| SNP | a23013c | N/A | 1 | 100.0000% | Spike | E484A |
| SNP | t23019c | N/A | 1 | 100.0000% | Spike | F486S |
| SNP | a23055g | N/A | 1 | 100.0000% | Spike | Q498R |
| SNP | a23063t | N/A | 1 | 100.0000% | Spike | N501Y |
| SNP | t23075c | N/A | 1 | 100.0000% | Spike | Y505H |
| SNP | a23403g | N/A | 1 | 100.0000% | Spike | D614G |
| SNP | c23525t | N/A | 1 | 100.0000% | Spike | H665Y |
| SNP | t23599g | N/A | 1 | 100.0000% | Spike | N679K |
| SNP | c23604a | N/A | 1 | 100.0000% | Spike | P681H |
| SNP | c23854a | N/A | 1 | 100.0000% | Spike | N764K |
| SNP | g23948t | N/A | 1 | 100.0000% | Spike | D796Y |
| SNP | a24424t | N/A | 1 | 100.0000% | Spike | Q954H |
| SNP | t24469a | N/A | 1 | 100.0000% | Spike | N969K |
| SNP | c25000t | N/A | 1 | 100.0000% | Spike | Silent mutation |
| SNP | c25416t | N/A | 1 | 100.0000% | ORF3a | Silent mutation |
| SNP | c25584t | N/A | 1 | 100.0000% | ORF3a | Silent mutation |
| SNP | c26060t | N/A | 1 | 100.0000% | ORF3a | T223I |
| SNP | c26270t | N/A | 1 | 100.0000% | Envelope | T9I |
| SNP | a26275g | N/A | 1 | 100.0000% | Envelope | T11A |
| SNP | c26577g | N/A | 1 | 100.0000% | Membrane | Q19E |
| SNP | g26709a | N/A | 1 | 100.0000% | Membrane | A63T |
| SNP | c26858t | N/A | 1 | 100.0000% | Membrane | Silent mutation |
| SNP | a27259c | N/A | 1 | 100.0000% | ORF6 | Silent mutation |
| SNP | g27382c | N/A | 1 | 100.0000% | ORF6 | D61L |
| SNP | a27383t | N/A | 1 | 100.0000% | | |
| SNP | t27384c | N/A | 1 | 100.0000% | | |
| SNP | c27807t | N/A | 1 | 100.0000% | ORF7b | Silent mutation |

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|---|---|---|---|---|---|---|
| SNP | a28271t | N/A | 1 | 100.0000% | Intergenic (ORF8/ Nucleocapsid) | Untranslated |
| SNP | c28311t | N/A | 1 | 100.0000% | Nucleocapsid | P13L |
| DEL | Δ28362- 28370 | N/A | -9 | 100.0000% | Nucleocapsid | ΔERS (amino acids 31-33) |
| SNP | g28881a | N/A | 1 | 100.0000% | Nucleocapsid | R203K |
| SNP | g28882a | N/A | 1 | 100.0000% | | |
| SNP | g28883c | N/A | 1 | 100.0000% | Nucleocapsid | G204R |
| SNP | A29510c | N/A | 1 | 100.0000% | Nucleocapsid | S413R |
| DEL | Δ29734- 29759 | N/A | -26 | 100.0000% | 3' UTR | Untranslated |
| DEL | Δ29902 | 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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