

SARS-Related Coronavirus 2, Isolate hCoV-19/Uganda/MUWRP-20200195568/2020 (Delta Variant)

Catalog No. NR-55607

Product Description:

Note: The strain designation on the vial label for lot 70045293 is incorrect. The correct strain designation is hCoV-19/Uganda/MUWRP-20200195568/2020. Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/Uganda/MUWRP-20200195568/2020 was collected from a nasal swab in Uganda on December 28, 2020. NR-55607 lot 70045293 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 4 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:

VETA2(1)VET(1)/C(1) (St. Jude Children's Research Hospital/BEI Resources); VETA2 = *Cercopithecus aethiops* kidney cells expressing transmembrane protease, serine 2 gene (TMPRSS2) and angiotensin-converting enzyme 2 (ACE2); VET = *Cercopithecus aethiops* kidney cells with TMPRSS2; C = Calu-3

Lot: 70045293

Manufacturing Date: 12JUN2021

BEI Resources is committed to ensuring digital accessibility for people with disabilities. This Certificate of Analysis contains complex tables and may not be fully accessible. Please let us know if you encounter accessibility barriers and a fully accessible document will be provided: E-mail: Contact@BEIResources.org. We try to respond to feedback within 24 hours.

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/Uganda/MUWRP-20200195568/2020 (GenBank: OL442161)	99.9% identity with SARS-CoV-2, hCoV-19/Uganda/MUWRP-20200195568/2020 (GenBank: OL442161)
Titer by TCID₅₀ Assay in Calu-3 Cells by Cytopathic Effect¹ (6 days at 37°C and 5% CO ₂)	Report results	8.9 × 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
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 (8.9 × 10⁴ per mL, 8.9 × 10⁴ per mL and 8.9 × 10⁴ 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.

/Heather Couch/
Heather Couch

07 DEC 2021

Program 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.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.



APPENDIX I: NGS Information for NR-55607 lot 70045293

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 GenBank OL442161.1 (see Table I below). Both NR-55607 lot 70045293 and GenBank OL442161.1 contained eighteen SNPs when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below). Additionally, NR-55607 lot 70045293 and GenBank MN908947 contained three SNPs when compared to GenBank OL442161.1 (see Table III below).

Table I: Variants with different nucleotides between NR-55607 lot 70045293 and GenBank OL442161.1 (SARS-CoV-2, isolate SARS-CoV-2_human_Uganda_MUWRP-20200195568_2020-P0)

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	t2200c	70	1	7.1429%	ORF1ab (nsp2)	Silent mutation
SNP	g10533t	185	1	28.1081%	ORF1ab (nsp5)	C160F
SNP	g11527t	151	1	5.2980%	ORF1ab (nsp6)	L185F
SNP	t11566a	87	1	5.7471%	ORF1ab (nsp6)	Silent mutation
SNP	t13339a	142	1	15.4930%	ORF1ab (nsp10)	N105K
SNP	t15357c	146	1	7.5342%	ORF1ab (nsp12)	Silent mutation
SNP	a25201g	91	1	5.4945%	S	Silent mutation
SNP	t25356g	137	1	22.6277%	S	L1265R
SNP	g27877t	300	1	98.3333%	ORF7b	C41F
SNP	c28695t	248	1	22.5806%	N	T141I

Table II: Variants with different nucleotides between NR-55607 lot 70045293 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	c4573t	n/a	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c8782t	n/a	1	100.0000%	ORF1ab (nsp4)	Silent mutation
SNP	c10747t	n/a	1	100.0000%	ORF1ab (nsp5)	Silent mutation
SNP	g11266t	n/a	1	100.0000%	ORF1ab (nsp6)	L98F
SNP	g11521t	n/a	1	100.0000%	ORF1ab (nsp6)	M183I
SNP	c16575t	n/a	1	100.0000%	ORF1ab (nsp13)	Silent mutation
SNP	c17745t	n/a	1	100.0000%	ORF1ab (nsp13)	Silent mutation
SNP	g21867t	n/a	1	100.0000%	S	R102I

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
SNP	c22000t	n/a	1	100.0000%	S	Silent mutation
SNP	c22033a	n/a	1	100.0000%	S	F157L
SNP	g22661t	n/a	1	100.0000%	S	V367F
SNP	g23401t	n/a	1	100.0000%	S	Q613H
SNP	c23604g	n/a	1	100.0000%	S	P681R
SNP	t24097c	n/a	1	100.0000%	S	Silent mutation
SNP	t28144c	n/a	1	100.0000%	ORF8	L84S
SNP	g28378c	n/a	1	100.0000%	N	Silent mutation
SNP	g28878a	n/a	1	100.0000%	N	S202N
SNP	g29742a	n/a	1	100.0000%	3'UTR	Untranslated

Table III: Variants with different nucleotides between NR-55607 lot 70045293 and GenBank OL442161.1 (SARS-CoV-2_human_Uganda_MUWRP-20200195568_2020), but matching nucleotides with GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Variant Type	Variant Position and Identified Alternative Base ²	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	g11230t_rev_g	131	1	6.8702%	ORF1ab (nsp6)	Silent mutation
SNP	t27254a_rev_t	189	1	99.4709%	ORF6	Silent mutation
SNP	g28167a_rev_g	149	1	6.0403%	ORF8	Silent mutation

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.

²rev = reversion. For example, g11230t represents the original mutation from "g" in Wuhan-Hu-1 to "t" in the provided reference genome. _rev_g represents the reversion back to g observed in the sample. (This variant is not represented as t11230g to avoid confusion regarding the wild-type nucleotide.)