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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52359

SARS-Related Coronavirus 2, Isolate England/02/2020

Catalog No. NR-52359

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate England/02/2020 was isolated from a COVID-19 patient during acute illness in February 2020 in England, United Kingdom. NR-52359 lot 70036177 was produced by infecting *Cercopithecus aethiops* kidney cells (Vero E6; ATCC® CRL-1586™) with the deposited material 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.

Passage History:

VE6(2)/VE6(2) (Public Health England/BEI Resources); VE6 = Vero E6 cells

Lot: 70036177 **Manufacturing Date: 01JUN2020**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 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	≥ 98% identity with SARS-CoV-2[1]
Titer by $TCID_{50}$ Assay in Vero E6 Cells by Cytopathic Effect[2] (5 days at 37°C and 5% CO_2)	Report results	2.8×10^6 $TCID_{50}$ per mL
Amplification of SARS-CoV-2 Sequence by RT-PCR	~ 950 base pair amplicon	~ 950 base pair amplicon
Sterility (33-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]Sequence information for SARS-CoV-2, England/02/2020 is not available in the NCBI database; nucleotide sequence obtained for NR-52359 lot 70036177 is 99.9% identical to SARS-CoV-2 isolate Wuhan-Hu-1, complete genome (GenBank: MN908947.3) and consistent with numerous SARS-CoV-2 strains.

[2]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.

[3]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/
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Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-52359 lot 70036177

Sequence analysis resulted in the discovery of six SNPs and two deletions when compared to SARS-CoV-2 isolate Wuhan-Hu-1, complete genome (GenBank: MN908947.3) (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52359_ 70036177 Sequence	Position in MN908947.3	Reported MN908947.3 Sequence	Identified Alternative Base	Quality	Variant Type	Frequency of Variant
673	685	AAAGTCATTTGACTTA	AGACTTA	61	Indel	0.108787
7737	7749	C	T	222	SNP	0.326667
8216	8228	C	T	221	SNP	0.283186
8770	8782	C	T	225	SNP	1.000000
18476	18488	T	C	225	SNP	1.000000
23585	23597	AATTCTCCTCGGCGGG CACGTAGTG	A	228	Indel	0.824121
28108	28144	T	C	225	SNP	1.000000
29560	29596	A	G	225	SNP	1.000000

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