

## Certificate of Analysis for NR-53565

#### SARS-Related Coronavirus 2, Isolate Canada/ON/VIDO-01/2020

### Catalog No. NR-53565

### **Product Description:**

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate Canada/ON/VIDO-01/2020 was isolated from a human patient sample collected on January 23, 2020 in Ontario, Canada. NR-53565 lot 70039216 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells (Vero E6; BEI Resources NR-596) and incubating in Eagle's Minimum Essential Medium (ATCC<sup>®</sup> 30-2003<sup>™</sup>) supplemented with 2% fetal bovine serum (ATCC<sup>®</sup> 30-2020<sup>™</sup>) for 4 days at 37°C with 5% CO<sub>2</sub>. Cell lysate and supernatant were clarified by centrifuging at 3000 rpm for 20 minutes at 4°C.

### Passage History:

VE(2)/VE(1) (National Microbiology Laboratory Canada/BEI Resources); VE = Vero E6 cells

Lot: 70039216 Manufacturing Date: 06OCT2020

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, Canada/ON/VIDO-01/2020 (GISAID: EPI_ISL_425177)	99.97% identity with SARS-COV- 2, Canada/ON/VIDO-01/2020 (GISAID: EPI_ISL_425177)		
Titer by TCID <sub>50</sub> Assay in Vero E6 Cells by Cytopathic Effect <sup>1</sup> (5 days at 37°C with 5% CO <sub>2</sub> )	Report results	2.8 × 10 <sup>5</sup> TCID <sub>50</sub> per mL		
Sterility (22-day incubation)				
Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>2</sup>	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		

<sup>1</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> 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<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> provides a measure of the titer (or infectivity) of a virus preparation. <sup>2</sup>Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 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-53565 lot 70039216

Sequence analysis using SBC v2.0 pipeline resulted in the discovery of eight SNPs when compared to the reference sequence from EPI\_ISL\_425177. Additionally, both the reference sequence EPI\_ISL\_425177 and NR-53565 lot 70039216 contained two SNPs when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-53565_ 70039216 Sequence	Position in EPI_ISL_ 425177 Reference Sequence	Position in MN908947 Wuhan- Hu-1 Sequence	Reported MN908947 Wuhan- Hu-1 Sequence	Reported EPI_ISL_ 425177 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
5051	5051	5084	Α	G	G	N/A	SNP	1	1.000000
5059	5059	5092	Т	Т	С	7004	SNP	1	0.101275
11050	11050	11083	G	G	Т	49314	SNP	1	0.553429
16424	16424	16457	С	С	Т	5740	SNP	1	0.072936
22173	22173	22206	Α	Α	G	41776	SNP	1	0.680457
23492	23492	23525	С	С	Т	28999	SNP	1	0.191518
23582	23582	23615	С	С	Α	49314	SNP	1	0.463395
28483	28483	28516	Т	Т	А	10905	SNP	1	0.069312
28821	28821	28854	С	Т	Т	N/A	SNP	1	1.000000
29806	29806	29839	А	А	G	832	SNP	1	0.074941

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