

SARS-Related Coronavirus 2, Isolate Germany/BavPat1/2020

Catalog No. NR-52370

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate Germany/BavPat1/2020 was isolated from a sputum sample from a mildly symptomatic adult male patient identified as Patient One in the Bavarian cluster on January 28, 2020 in Bavaria, Germany. NR-52370 lot 70036595 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% CO₂.

Passage History:

VE6(3)/VE6(2) (Bundeswehr Institute of Microbiology/BEI Resources); VE6 = Vero E6 cells

Lot: 70036595

Manufacturing Date: 12JUN2020

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, isolate Human/DEU/BavPat1-ChVir929/2020 (GenBank: MT270101.1 and GISAID: EPI_ISL_406862)	99.93% identity with SARS-CoV-2, isolate Human/DEU/BavPat1-ChVir929/2020 (GenBank: MT270101.1 and GISAID: EPI_ISL_406862)
Titer by TCID₅₀ Assay in Vero E6 Cells by Cytopathic Effect¹ (5 days at 37°C and 5% CO ₂)	Report results	2.8 × 10 ⁶ TCID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ² 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 and 5% CO ₂	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.

²Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/

Heather Couch

17 AUG 2020

Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-52370 lot 70036595

Sequence analysis resulted in the discovery of four SNPs and one deletion when compared to the reference sequence from GISAID EPI_ISL_406862. Additionally, both the reference sequence GISAID EPI_ISL_406862 and NR-52370_70036595 contained three 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-52370_70036595 Sequence	Position in EPI_ISL_406862 Reference Sequence	Position in MN908947 Sequence	Reported MN908947 Sequence	Reported EPI_ISL_406862 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
187	187	241	C	T	T	n/a	SNP	1	1.0000000
2983	2983	3037	C	T	T	n/a	SNP	1	1.0000000
5752	5752	5806	C	C	T	222	SNP	1	0.6200397
13939	13939	13993	G	G	T	228	SNP	1	0.9842342
21952	21952	22006	C	C	A	51	SNP	1	0.1939655
23349	23349	23403	A	G	G	n/a	SNP	1	1.0000000
23541	23541	23595	CTAATTCT CCTTCGG CGGGCAC	CTAATTCT CCTTCGGC GGGCAC	C	n/a	Indel	20	0.5647637
28797	28814	28868	C	C	T	228	SNP	1	0.9840286