

SARS-Related Coronavirus 2, Isolate Italy-INMI1

Catalog No. NR-52284

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate Italy-INMI1 was isolated from sputum of a patient with a respiratory illness who had recently returned from travel to the affected region of China and developed clinical disease (COVID-19) in January 2020 in Rome, Italy. NR-52284 lot 70034928 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 4 days at 37°C with 5% CO₂.

Passage History:

VE6(2)/VE6(1) (Lazzaro Spallanzani National Institute for Infectious Diseases/BEI Resources); VE6 = Vero E6 cells

Lot: 70034928

Manufacturing Date: 07APR2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 820 nucleotides)	≥ 98% identity with SARS-CoV-2, isolate Italy-INMI1 (GenBank: MT066156.1)	100% identity with SARS-CoV-2, isolate Italy-INMI1 (GenBank: MT066156.1)
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 Italy-INMI1 (GenBank: MT066156.1)	99.99% identity with SARS-CoV-2, isolate Italy-INMI1 (GenBank: MT066156.1)
Titer by TCID₅₀ Assay in Vero E6 Cells by Cytopathic Effect¹ (4 days at 37°C and 5% CO ₂)	Report results	1.6 × 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, 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.

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

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APPENDIX I: NGS Information for NR-52284 lot 70034928

Sequence analysis resulted in the discovery of three SNPs and one deletion when compared to GenBank MT066156.1 (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52284_70034928 Sequence	Position in MT066156.1	Reported MT066156.1 Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
2262	2269	T	A	228	SNP	1	0.8584071
11076	11083	N	T	225	SNP	1	1.0000000
23587	23594	ACTAATTCTCCTCGGCG GGCACGTAGTGTAGCTA	ACTA	222	Indel	30	0.6113360
23599	23606	C	T	129	SNP	1	0.2089552