

Certificate of Analysis for NR-52506

Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-WI1/2020

Catalog No. NR-52506

This reagent is the property of the U.S. Government.

Product Description:

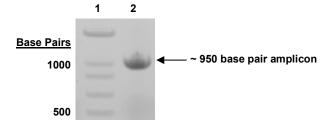
Genomic RNA was extracted from a preparation of cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero E6; ATCC® CRL-1586™) infected with severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA-WI1/2020 (BEI Resources NR-52384 lot 70034881), using QIAamp® Viral RNA Mini Kit (Qiagen 52904). The viral genomic RNA is in a background of cellular nucleic acid and carrier RNA.

Lot: 70035257 Manufacturing Date: 28MAY2020

TEST	SPECIFICATIONS	RESULTS 100% identity with SARS-CoV-2, isolate USA-WI1/2020 (GenBank: MT039887.1)		
Sequencing of Species-Specific Region (~ 870 nucleotides)	≥ 98% identity with SARS-CoV-2, isolate USA-WI1/2020 (GenBank: MT039887.1)			
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 USA-WI1/2020 (GenBank: MT039887.1)	99.96% identity with SARS-CoV-2, isolate USA-WI1/2020 (GenBank: MT039887.1)		
Functional Activity by RT-PCR Amplification ¹	~ 950 base pair amplicon	~ 950 base pair amplicon (Figure 1)		
Genome Copy Number Using BioRad QX200 Droplet Digital PCR (ddPCR™) System (Post vial; 9 replicates)	Report results	7.5 × 10 ⁷ genome equivalents/mL		
Virus Inactivation (14 Days, 2 Passages) 10% of total yield inoculated on Vero E6 cells and evaluated for cytopathic effect ²				
Passage 1	No viable virus detected	No viable virus detected		
Passage 2	No viable virus detected	No viable virus detected		
Virus Inactivation (14 Days, 2 Passages) 100% of total yield inoculated on Vero E6 cells and evaluated for cytopathic effect ²				
Passage 1	No viable virus detected	No viable virus detected		
Passage 2	No viable virus detected	No viable virus detected		

Amplified using iTaq™ Universal SYBR Green One-step Kit (Bio-Rad 172-5151) with 5 µL of NR-52506 in a 50 µL reaction

Figure 1: Functional Activity of NR-52506 by RT-PCR Amplification



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder Lane 2: PCR product from 1 µL of NR-52506

BEI Resources www.beiresources.org E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898

²Supernatant was tested by qPCR to confirm absence of CPE and no evidence of replicative RNA was detected. Samples from both passages were tested by qPCR at the end of day 14 of the passages.



Certificate of Analysis for NR-52506

/Heather Couch/ Heather Couch

04 SEP 2020

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.

Support Provided by NIAID

APPENDIX I: NGS Information for NR-52506 lot 70035257

Sequence analysis resulted in the discovery of two SNPs and a single deletion when compared to GenBank MT039887.1 (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52506_ 70035257 Sequence	Position in MT039887.1	Reported MT039887.1 Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
11831	11835	С	Т	225	SNP	1	1.0000000
22199	22203	A	G	228	SNP	1	0.9327731
23609	23613	GTAGTGTAGCTAGT	GTAGT	222	Indel	9	0.5271967

BEI Resources www.beiresources.org E-mail: contact@beiresources.org
Tel: 800-359-7370

Fax: 703-365-2898