

Certificate of Analysis for NR-55351

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/MD-HP01542/2021 (Lineage B.1.351), in *Homo sapiens* Lung Adenocarcinoma (Calu-3) Cells, Gamma-Irradiated

Catalog No. NR-55351

Product Description:

NR-55351 is a crude preparation of cell lysate and supernatant from *Homo sapiens* lung adenocarcinoma epithelial cells (Calu-3; ATCC® HTB-55 $^{\text{TM}}$) infected with SARS-CoV-2, isolate hCoV-19/USA/MD-HP01542/2021 that was gamma-irradiated (5 × 10⁶ RADs) on dry ice, followed by sonication to clarify the supernatant prior to vialing.

The starting material (lot 70043444) was produced by incubating *Homo sapiens* lung adenocarcinoma epithelial cells (Calu-3; ATCC® HTB-55TM) in Eagle's Minimum Essential Medium (ATCC® 30-2003TM) supplemented with 2% fetal bovine serum (ATCC® 30-2020TM) for 3 days at 37°C with 5% CO₂. Titer of spin clarified source material was 1.1 × 10⁵ TCID₅₀ per mL by TCID₅₀ assay in Calu-3 cells, determined by cytopathic effect in 8 days at 37°C with 5% CO₂, and sterility of the preparation was confirmed.

Note: The vial label is incorrect. The correct name of the organism is SARS-Related Coronavirus 2, Isolate hCoV-19/USA/MD-HP01542/2021 (Lineage B.1.351).

Lot: 70045298 Manufacturing Date: 22JUN2021

TEST	SPECIFICATIONS	RESULTS
Genome Copy Number Using BioRad QX200 Droplet Digital PCR (ddPCR™) System¹ (9 replicates)	Report results	7.36 × 10 ⁷ genome copies per mL
Virus Inactivation 10% of total bulk gamma-irradiated preparation inoculated on Vero E6 with high expression of ACE2 cells and evaluated for cytopathic effect and expression of viral antigen by indirect immunofluorescence assay ^{2,3}	No viable virus detected	No viable virus detected

The genome copy number reported is obtained using Qiagen RNA extraction kit (Cat 52906).

/Heather Couch/

Heather Couch 30 SEP 2021

Program Manager or designee, ATCC Federal Solutions

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²The gamma-irradiated virus preparation was plated on Vero E6 cells and incubated for 14 days at 37°C and 5% CO₂; cell lysate and supernatant from these cultures were blind passaged on fresh monolayers of Vero E6 cells and again incubated for 14 days at 37°C and 5% CO₂.

³Performed at University of Texas Medical Branch, Galveston, Texas, USA