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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-53530

Human Coronavirus, NL63, Heat Inactivated

Catalog No. NR-53530

Product Description:

NR-53530 is a preparation of human coronavirus (HCoV), NL63 that has been inactivated by heating to 65°C for 30 minutes, followed by 8-fold dilution prior to vialing.

The starting material (BEI Resources NR-470 lot 70037857) was produced by infecting *Macaca mulatta* kidney epithelial cells (LLC-MK2; ATCC® CCL-7.1™) in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 7 days at 34°C with 5% CO_2. Titer of NR-470 was 1.6×10^4 $TCID_{50}$ per mL by $TCID_{50}$ assay in LLC-MK2 cells, determined by cytopathic effect in 7 days at 34°C with 5% CO_2, and sterility of the preparation was confirmed.[1]

Lot: 70036555 **Manufacturing Date: 11DEC2020**

TEST	SPECIFICATIONS	RESULTS
Genome Copy Number Using BioRad QX200 Droplet Digital PCR (ddPCR™) System[2]	Report results	5.51×10^7 genome equivalents per mL[3]
Virus Inactivation 10% of total bulk heat-treated preparation inoculated on LLC-MK2 cells and evaluated for cytopathic effect and presence of viral RNA by qRT-PCR[4]	No viable virus detected	No viable virus detected

[1]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.
[2]The genome copy number reported is obtained using Qiagen RNA extraction kit (Cat 52904).
[3]Two vials of NR-53530 lot 70036555 were used for RNA extraction. Average of the results obtained from nine replicates from each vial is reported.
[4]The inactivated virus preparation was plated on LLC-MK2 cells and incubated for 14 days at 34°C and 5% CO_2; cell lysate and supernatant from these cultures were blind passaged on fresh monolayers of LLC-MK2 cells and again incubated for 14 days at 34°C and 5% CO_2. Samples from both passages were also tested by qPCR at the end of day 14 of the passages.

/Heather Couch/
Heather Couch 28 APR 2021
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.

Support Provided by NIAID

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