b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-470

Human Coronavirus, NL63

Catalog No. NR-470

Product Description:

Human coronavirus (HCoV), NL63 was isolated in 2003 from nasopharyngeal aspirate of human infant with acute respiratory disease in Amsterdam. NR-470 lot 70037857 was produced by infecting *Macaca mulatta* kidney epithelial cells (LLC-MK2; ATCC® CCL-7.1™) with BEI Resources seed lot 3945808 and incubating 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.

Passage History:

MK(8)/MK(2) (Prior to deposit at BEI Resources/BEI Resources); MK = LLC-MK2 cells

Lot: 70037857 **Manufacturing Date: 24JUL2020**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in LLC-MK2 Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 960 nucleotides)	≥ 98% identity with HCoV, NL63 (GenBank: AY567487.2)	100% identity with HCoV, NL63 (GenBank: AY567487.2)
Titer by $TCID_{50}$ Assay in LLC-MK2 Cells by Cytopathic Effect[1] (7 days at 34°C with 5% CO_2)	Report results	1.6×10^4 $TCID_{50}$ per mL
Amplification of HCoV Sequence by RT-PCR	~ 1060 base pair amplicon	~ 1060 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[2]	No growth	No growth
Trypticase Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C, aerobic	No growth	No growth
Mycoplasma Contamination		
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

[1]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint 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]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/
Heather Couch 23 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

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898