

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 70033870 was produced by infecting *Macaca mulatta* kidney epithelial cells (LLC-MK2; ATCC® CCL-7.1™) and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 6 days at 34°C with 5% CO₂.

Passage History:

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

Lot: 70033870

Manufacturing Date: 19MAR2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in LLC-MK2 Cells	Cell lysis	Cell lysis
Identification by Sequencing (~ 970 nucleotides)	≥ 98% identity with HCoV, NL63 (GenBank: AY567487.2)	100% identity with HCoV, NL63 (GenBank: AY567487.2)
Titer by TCID₅₀ Assay in LLC-MK2 Cells by Cytopathic Effect¹ (8 days at 34°C with 5% CO ₂)	Report results	1.6 × 10 ⁵ 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 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 and 5% CO ₂	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.

/Heather Couch/
Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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