

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 70047796 was produced by infecting derivative *Macaca mulatta* kidney epithelial (LLC-MK2; ATCC® CCL-7.1™) with seed material (BEI Resources lot 3946804) 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% CO₂.

Passage History:

MK(8)/MK(3) (Prior to deposit at BEI Resources/BEI Resources); MK = LLC-MK2 cells

Lot: 70047796 Manufacturing Date: 12OCT2021

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TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in LLC-MK2 Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 940 nucleotides)	≥ 98% identity with HCoV, NL63 (GenBank: AY567487.2)	100% identity with HCoV, NL63 (GenBank: AY567487.2)
Titer by TCID₅ Assay in LLC-MK2 Cells by Cytopathic Effect¹ (8 days at 34°C and 5% CO₂)	Report results	1.6 × 10 ⁵ TCID ₅₀ per mL
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

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. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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Program Manager or designee, ATCC Federal Solutions

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