

Certificate of Analysis for NR-43000

Recombinant Murine Coronavirus, icA59

Catalog No. NR-43000

Product Description:

NR-43000 is a recombinant murine coronavirus that was produced using a vaccinia virus-based reverse genetics system, and derived from cloned, full-length MHV-A59 cDNA. NR-43000 lot 70033869 was produced by infecting *Mus musculus* liver epithelial cells (NCTC clone 1469 cells; ATCC[®] CCL-9.1[™]) and incubating in Dulbecco's Modified Eagle Medium (ATCC[®] 30-2002[™]) supplemented with 2% heat-inactivated horse serum (Gibco[®] 26050-070) for 2 days at 37°C with 5% CO₂.

Passage History:

17Cl-1(2 or 3)/NCTC(2) (Prior to deposit at BEI Resources/BEI Resources); 17Cl-1 = Mouse fibroblast 17Cl-1 cells; NCTC = NCTC clone 1469 cells

Lot: 70033869 Manufacturing Date: 27MAR2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in NCTC Clone 1469 Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 660 nucleotides)	≥ 98% identity with MHV-A59, icA59 (GenBank: KF268337.1)	100% identity with MHV-A59, icA59 (GenBank: KF268337.1)
Titer by TCID ₅₀ Assay in NCTC Clone 1469 Cells by Cytopathic Effect ¹ (4 days at 37°C with 5% CO ₂)	Report results	8.9 × 10 ⁷ TCID ₅₀ per mL
Amplification of Murine Coronavirus Sequence by RT-PCR	~ 680 base pair amplicon	~ 680 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 and 5% CO ₂	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. Handbook of Microbiological Media. 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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