

Recombinant Murine Coronavirus, icA59-ns2dm

Catalog No. NR-43001

Product Description:

NR-43001 lot 70037386 was produced by infecting *Mus musculus* liver epithelial cells (NCTC Clone 1469; ATCC® CCL-9.1™) and incubating in Dulbecco's Modified Eagle's Medium (ATCC® 30-2002™) supplemented with 10% fetal bovine serum (ATCC® 30-2020™) for 1 day at 37°C with 5% CO₂.

Passage History:

Mouse fibroblasts(2)/NCTC Clone 1469(2) (Prior to deposit at BEI Resources/BEI Resources)

Lot: 70037386

Manufacturing Date: 25JAN2021

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in NCTC Clone 1469 Cells	Cell enlargement and detachment	Cell enlargement and detachment
Sequencing of Species-Specific Region (~ 650 nucleotides)	≥ 98% identity with murine coronavirus, icA59-ns2dm (GenBank: KF268339) ^{1,2}	100% identity with murine coronavirus, icA59-ns2dm (GenBank: KF268339) ^{1,2}
Titer by TCID₅₀ Assay in NCTC Clone 1469 Cells by Cytopathic Effect³ (6 days at 37°C with 5% CO ₂)	Report results	1.6 × 10 ⁷ TCID ₅₀ per mL
Amplification of murine CoV Sequence by RT-PCR	~ 690 base pair amplicon	~ 690 base pair amplicon
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ⁴ 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, aerobic	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

¹Note that the nomenclature used for this virus in the NCBI database differs from that used here.

²Sequence analysis at BEI Resources confirmed the presence of the H46A and H126R mutations described in Roth-Cross, J. K., et al. "Organ-Specific Attenuation of Murine Hepatitis Virus Strain A59 by Replacement of Catalytic Residues in the Putative Viral Cyclic Phosphodiesterase ns2." *J. Virol.* 83 (2009): 3743-3753. PubMed: 19176619.

³The 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.

⁴Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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