

Recombinant Murine Coronavirus MHV-A59-MHV2Spike with Enhanced Green Fluorescent Protein (eGFP)

Catalog No. NR-53717

Product Description:

Murine coronavirus (MHV), isolate MHV-A59-MHV2Spike-eGFP is a recombinant MHV-A59 virus with spike genes from strain MHV-2 in which open reading frame 4 (ORF4) was replaced by a gene encoding the enhanced green fluorescent protein (eGFP). NR-53717 lot 70038110 was produced by infecting murine 17CI-1 cells (BEI Resources NR-53719) in Dulbecco's Modified Eagle's Medium (ATCC® 30-2002™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 1 day at 37°C with 5% CO₂.

Passage History:

X(?)/C(2) (Prior to deposit at BEI Resources/BEI Resources); X = Unknown; C = 17CI-1 cells

Lot: 70038110

Manufacturing Date: 04DEC2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in 17CI-1 Cells	Cell rounding and detachment	Cell rounding and detachment
Identification by eGFP Expression in 17CI-1 Cells	Fluorescence observed	Fluorescence observed
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform (~ 24000 nucleotides) (~ 4060 nucleotides)	≥ 98% identity with MHV, A59 (GenBank: AY700211.1) ≥ 98% identity with MHV spike protein mRNA, complete cds (GenBank: U72635.1)	99.9% identity with MHV, A59 (GenBank: AY700211.1) 99.9% identity with MHV spike protein mRNA, complete cds (GenBank: U72635.1)
Titer by TCID ₅₀ Assay in 17CI-1 Cells by Cytopathic Effect and eGFP Expression ¹ (4 days at 37°C and 5% CO ₂)	Report results	1.6 × 10 ⁹ TCID ₅₀ per mL
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, 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

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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