

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

**Catalog No. NR-53716**

**Product Description:**

Murine coronavirus (MHV), isolate MHV-A59-eGFP is a recombinant MHV-A59 virus in which open reading frame 4 (ORF4) was replaced by a gene encoding the enhanced green fluorescent protein (eGFP). NR-53716 lot 70038108 was produced by infecting murine 17Cl-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% CO<sub>2</sub>.

**Passage History:**

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

**Lot: 70038108**

**Manufacturing Date: 04DEC2020**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in 17Cl-1 Cells	Cell rounding and detachment	Cell rounding and detachment
Identification by eGFP Expression in 17Cl-1 Cells	Fluorescence observed	Fluorescence observed
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform (~ 31040 nucleotides)	≥ 98% identity with MHV, A59 (GenBank: AY700211.1)	99.9% identity with MHV, A59 (GenBank: AY700211.1)
Titer by TCID <sub>50</sub> Assay in 17Cl-1 Cells by Cytopathic Effect and eGFP Expression <sup>1</sup> (4 days at 37°C and 5% CO <sub>2</sub> )	Report results	1.6 × 10 <sup>8</sup> TCID <sub>50</sub> per mL
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>2</sup> 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
<b>Mycoplasma Contamination</b> 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

<sup>1</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> 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% (LD<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> provides a measure of the titer (or infectivity) of a virus preparation.

<sup>2</sup>Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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