

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

Catalog No. NR-53716

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

Virus Classification: *Coronaviridae, Betacoronavirus*

Species: Murine coronavirus [formerly murine hepatitis virus (MHV)]

Isolate: MHV-A59-eGFP

Original Source: 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).^{1,2}

Comments: NR-53716 is a virus preparation made using targeted recombination and selection for MHV with stable and efficient expression of the gene encoding eGFP. The eGFP gene was inserted into the MHV genome in place of the nonessential gene ORF4 in mildly neurovirulent strain, MHV-A59.² eGFP is a modified version of GFP gene designed for brighter fluorescence, in which the codon utilization has been maximized for translation in eukaryotic cells.^{3,4} Expression of eGFP is stable over multiple passages *in vitro* and at a level high enough to be readily detected in cultured cells and in the central nervous system of infected animals.²

Material Provided:

Each vial contains approximately 1.0 mL of cell lysate and supernatant from murine 17Cl-1 cells infected with MHV, isolate MHV-A59-eGFP.

Note: If homogeneity is required for your intended use, please purify prior to initiating work.

Packaging/Storage:

NR-53716 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen and should be stored at -60°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended. Freeze-thaw cycles should be avoided.

Growth Conditions:

Host: Murine 17Cl-1 cells (BEI Resources NR-53719)

Growth Medium: Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 4 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, and 1500 mg/L sodium

bicarbonate supplemented with 2% fetal bovine serum or equivalent

Infection: Cells should be 70% to 80% confluent

Incubation: 2 to 4 days at 37°C and 5% CO₂

Cytopathic Effect: Cell rounding and sloughing

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Recombinant Murine Coronavirus MHV-A59 with Enhanced Green Fluorescent Protein (eGFP), NR-53716."

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. Weiss, S., Personal Communication.
2. Sarma, J. D., et al. "Enhanced Green Fluorescent Protein Expression May be Used to Monitor Murine Coronavirus Spread *in vitro* and in the Mouse Central Nervous System." J. Neurovirol. 8 (2002): 381-391. PubMed: 12402164.
3. Cormack, B. P., R. H. Valdivia and S. Falkow. "FACS-Optimized Mutants of the Green Fluorescent Protein (GFP)." Gene 173 (1996): 33-38. PubMed: 8707053.
4. Haas, J., E. C. Park and B. Seed. "Codon Usage Limitation in the Expression of HIV-1 Envelope Glycoprotein." Curr. Biol. 6 (1996): 315-324. PubMed: 8805248.

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