

Vector pMCSG53 Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Structural Protein 1 Gene

Catalog No. NR-52899

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Contributor:

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Manufacturer:

BEI Resources

Product Description:

The non-structural protein 1 (nsp1) gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: [MN908947](#)) was codon optimized and cloned into the [pMCSG53](#) plasmid.^{1,2} pMCSG53 is an *Escherichia coli* (*E. coli*) expression vector that contains an N-terminal hexa-histidine tag, followed by a tobacco etch virus (TEV) protease recognition site prior to the insert coding sequence, resulting in the expression of a cleavable histidine-tagged protein.³ It also contains tRNA genes covering rare codons for arginine (AGG/AGA) and isoleucine (AUA) to improve expression in *E. coli*. The beta-lactamase gene, *bla*, provides transformant selection through ampicillin resistance in *E. coli*. The resulting size of the plasmid is approximately 5330 base pairs. The complete plasmid sequence and map are provided on the BEI Resources webpage. The plasmid was produced in *E. coli* and extracted.

Nsp1 is produced from the SARS-CoV-2 ORF1a polyprotein, triggering host mRNA degradation by processes such as binding to the 40S ribosomal subunit, resulting in suppression of antiviral signaling such as RIG-I-dependent innate immune responses.^{4,5} Nsp1 also protects viral transcripts, although this mechanism is under study.⁶

Material Provided:

Each vial contains plasmid DNA in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The DNA concentration and volume provided are shown on the Certificate of Analysis. The vial should be centrifuged prior to opening. **Note:** The contents of the vial should be used to replicate the plasmid in *E. coli* prior to expression studies.

Packaging/Storage:

NR-52899 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -20°C or colder immediately upon arrival. Freeze-thaw cycles should be minimized.

Citation:

Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: Vector pMCSG53 Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Structural Protein 1 Gene, NR-52899.”

Biosafety Level: 1

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](#). 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmb15/index.htm.

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References:

1. Joachimiak, A., Personal Communication.
2. Wu, F., et al. “A New Coronavirus Associated with Human Respiratory Disease in China.” *Nature* 579 (2020): 265-269. PubMed: 32015508.

3. Eschenfeldt, W. H., et al. "New LIC Vectors for Production of Proteins from Genes Containing Rare Codons." J. Struct. Funct. Genomics 14 (2013): 135-144. PubMed: 24057978.
4. Huang, C., et al. "SARS Coronavirus Nsp1 Protein Induces Template-Dependent Endonucleolytic Cleavage of mRNAs: Viral mRNAs Are Resistant to Nsp1-Induced RNA Cleavage." PloS Pathog. 7 (2011): e1002433. PubMed: 22174690.
5. Thoms, M., et al. "Structural Basis for Translational Shutdown and Immune Evasion by the Nsp1 Protein of SARS-CoV-2." Science (2020): *in press*. PubMed: 32680882.
6. Rodriguez, W., et al. "Fated for Decay: RNA Elements Targeted by Viral Endonucleases." Semin. Cell Dev. Biol. S1084-9521 (2020): 30200-30209. PubMed: 32522410.

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