

Product Information Sheet for NR-52897

Vector pMCSG53 Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Papain-Like Protease Gene

Catalog No. NR-52897

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Contributor:

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Manufacturer:

BEI Resources

Product Description:

The papain-like protease [PLpro; amino acids 746 to 1060 of non-structural protein 3 (NSP3); GenPept: [YP_009725299](#)] 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 5740 base pairs. The complete plasmid sequence and map are provided on the BEI Resources webpage. The plasmid was produced in *E. coli* and extracted.

PLpro is located within the NSP3 domain of the SARS-CoV-2 ORF1ab polyprotein. PLpro is a cysteine protease that, together with the 3C-like protease (3CLpro), processes the viral polyproteins in preparation for viral replication. It is also involved in viral evasion of the host innate immune responses via reversal of ubiquitin and interferon-stimulated gene 15 (ISG15) post-translational modifications from host-cell proteins.^{4,5}

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-52897 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 Papain-Like Protease Gene, NR-52897."

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/bmbl5/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. Báez-Santos, Y. M., S. E. St. John and A. D. Mesecar. "The SARS-Coronavirus Papain-Like Protease: Structure, Function and Inhibition by Designed Antiviral Compounds." Antiviral Res. 115 (2015): 21-38. PubMed: 25554382.
5. Freitas, B. T., et al. "Characterization and Noncovalent Inhibition of the Deubiquitinase and deISGylase Activity of SARS-CoV-2 Papain-Like Protease." ACS Infect. Dis. (2020): *in press*. PubMed: 32428392.

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