Vector pMCSG53 Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Structural Protein 10 Gene

Catalog No. NR-52425

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For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

The non-structural protein 10 (nsp10) 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. It also contains tRNA genes covering rare codons for Arg (AGG/AGA) and Ile (AUA) to improve expression in *E. coli*. The beta-lactamase gene, *bla*, provides transformant selection through ampicillin resistance in *E. coli*.[3,4] The resulting size of the plasmid is approximately 5230 base pairs. The complete plasmid sequence and map are provided on the BEI Resources webpage. The plasmid was produced in *E. coli* and extracted.

Non-structural protein 10 is a non-structural protein located within the SARS-CoV-2 ORF1ab and is required for viral replication. It has been shown to stimulate the activity of two methyl transferases, Nsp14 and Nsp16, resulting in the production of a viral RNA cap that prevents the activation of the host innate immune response.[5,6]

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-52425 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 10 Gene, NR-52425, contributed by the Center for Structural Genomics of Infectious Diseases under HHSN272201700060C."

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. 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. Satchell, K. J., 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. Stols, L., et al. "A New Vector for High-Throughput, Ligation-Independent Cloning Encoding a Tobacco Etch Virus Protease Cleavage Site." Protein Expr. Purif. 25 (2002): 8-15. PubMed: 12071693.
4. 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.
5. Bouvet, M., et al. "Coronavirus Nsp10, a Critical Co-Factor for Activation of Multiple Replicative Enzymes." J. Biol. Chem. 289 (2014): 25783-25796. PubMed: 25074927.
6. Wang, Y., et al. "Coronavirus Nsp10/Nsp16 Methyltransferase can be Targeted by Nsp10-Derived Peptide *in vitro* and *in vivo* to Reduce Replication and Pathogenesis." J. Virol. 89 (2015): 8416-8427. PubMed: 26041293.

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