

Vector pET-28a(+) Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Structural Protein 14 Gene

Catalog No. NR-53505

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Contributor:

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Manufacturer:

BEI Resources

Product Description:

The non-structural protein 14 (nsp14) gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: [MN908947](#)) was codon optimized, tagged with a C-terminal hexa-histidine tag and cloned into the [pET-28a\(+\)](#) plasmid.^{1,2} The kanamycin resistance gene, *aph*, provides transformant selection through kanamycin resistance in *Escherichia coli* (*E. coli*). The resulting size of the plasmid is approximately 6830 base pairs. The complete plasmid sequence and map are provided on the BEI Resources webpage. The plasmid was produced in *E. coli* and extracted.

NSP14 is a multifunctional protein located in the ORF1b polyprotein. The amino-terminal domain is a 3'-5' exoribonuclease (ExoN) involved in a replicative mismatch repair system.^{3,4} The carboxy-terminal domain is a cap (guanine-N7) methyltransferase that, together with NSP10 and NSP16, generates mRNA 5'-terminal caps.^{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-53505 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 pET-28a(+) Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Structural Protein 14 Gene, NR-53505."

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. Van Voorhis, W., 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. Denison, M. R., et al. "Coronaviruses: An RNA Proofreading Machine Regulates Replication Fidelity and Diversity." RNA Biol. 8 (2011): 270-279. PubMed: 21593585.
4. Subissi, L., et al. "SARS-CoV ORF1b-Encoded Nonstructural Proteins 12-16: Replicative Enzymes as Antiviral Targets." Antiviral Res. 101 (2014): 122-130. PubMed: 24269475.
5. Jin, X., et al. "Characterization of the Guanine-N7 Methyltransferase Activity of Coronavirus Nsp14 on Nucleotide GTP." Virus Res. 176 (2013): 45-52. PubMed: 23702198.

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