

**Vector pET-28a(+) Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Open Reading Frame 3a Gene**

**Catalog No. NR-53509**

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**Contributor:**

Wesley Van Voorhis, M.D., Ph.D., Professor, Department of Medicine, Division of Allergy and Infectious Diseases (AID), Director, Center for Emerging and Re-emerging Infectious Diseases (CERID), and Co-Principal Investigator, Seattle Structural Genomics Center for Infectious Disease (SSGICD), University of Washington, Seattle, Washington, USA

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**Manufacturer:**

BEI Resources

**Product Description:**

The open reading frame 3a (orf3a) gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: [MN908947](#)) was codon optimized, tagged with a tobacco etch virus (TEV) cleavable N-terminal hexa-histidine tag and cloned into the [pET-28a\(+\)](#) plasmid.<sup>1,2</sup> The kanamycin resistance gene, *aph*, provides transformant selection through kanamycin resistance in *Escherichia coli* (*E. coli*). The resulting size of the plasmid is approximately 6140 base pairs. The complete plasmid sequence and map are provided on the BEI Resources webpage. The plasmid was produced in *E. coli* and extracted.

ORF3a is an ion channel protein which localizes to the Golgi complex and contributes to viral replication and virulence. ORF3a activates the NLRP3 inflammasome in SARS-CoV, part of the innate immune antiviral response, by inducing the proinflammatory cytokine interleukin-1 $\beta$ , resulting in apoptosis and cytokine storm promotion.<sup>3,4,5</sup>

**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-53509 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 Open Reading Frame 3a Gene, NR-53509.”

**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](http://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. Yoshimoto, F. "The Proteins of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2 or n-COV19), the Cause of COVID-19." Protein J. 39 (2020): 198-216. PubMed: 32447571.
4. Siu, K. -L., et al. "Severe Acute Respiratory Syndrome Coronavirus ORF3a Protein Activates the NLRP3 Inflammasome by Promoting TRAF3-Dependent Ubiquitination of ASC." FASEB J. 33 (2019): 8865-8877. PubMed: 31034780.
5. Ren, Y., et. al. "The ORF3a Protein of SARS-CoV-2 Induces Apoptosis in Cells." Cell Mol. Immunol. 17 (2020): 881-883. PubMed: 32555321.

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