

**Vector pLVX-EF1 $\alpha$ -IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Open Reading Frame 8 Gene**

**Catalog No. NR-52972**

**For research use only. Not for use in humans.**

**Contributor:**

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**Manufacturer:**

BEI Resources

**Product Description:**

Note: The vial label indicates this product contains a TST tag. This nomenclature refers to a 2X Strep tag.<sup>1,2</sup> This product does not express the Twin-Strep-tag<sup>®</sup> that is commonly referred to as a TST tag.

The open reading frame 8 (orf8) gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), USA-WA1/2020 (GenBank: [MN985325](#)) was codon optimized and modified by the addition of a C-terminal 2X Strep tag and cloned into the [pLVX-EF1 \$\alpha\$ -IRES-Puro](#) lentiviral expression plasmid.<sup>1,2,3</sup> The vector contains an internal ribosomal entry site (IRES) that allows a gene-of-interest and a puromycin resistance gene to be simultaneously co-expressed from a single mRNA transcript. Expression of the transcript is driven by the human elongation factor 1 alpha (EF1 $\alpha$ ) promoter. The beta-lactamase gene, *bla*, provides transformant selection through ampicillin resistance in *Escherichia coli* (*E. coli*) and the puromycin resistance gene, *pac*, provides transformant selection through puromycin resistance in eukaryotic cells. The resulting size of the plasmid is approximately 9280 base pairs. NR-52972 can be used for transient expression and lentivirus generation.<sup>1</sup> The complete plasmid sequence and map are provided on the BEI Resources webpage. The plasmid was produced in *E. coli* and extracted.

The biological function of the SARS-CoV-2 accessory protein ORF8 remains unclear, yet recent studies indicate ORF8 is strongly immunogenic and remarkably divergent, showing the least homology (< 20%) with SARS-CoV among all viral proteins.<sup>4,5</sup> A study suggested that ORF8 mediates immune evasion by downregulating MHC-I in cells.<sup>6</sup> ORF8 is a hotspot for coronavirus mutation, and the clinical effect of deletions in this region may alter immune response to SARS-CoV-2.<sup>7,8</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 mammalian expression studies.

**Packaging/Storage:**

NR-52972 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 pLVX-EF1 $\alpha$ -IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Open Reading Frame 8 Gene, NR-52972.”

**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:**

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6. Zhang, Y., et al. "The ORF8 Protein of SARS-CoV-2 Mediates Immune Evasion through Potently Downregulating MHC-I." *bioRxiv* (2020): *in press*. doi: <https://doi.org/10.1101/2020.05.24.111823>.
7. Su, Y. C. F., et al. "Discovery and Genomic Characterization of a 382-Nucleotide Deletion in ORF7b and ORF8 during the Early Evolution of SARS-CoV-2." *mBio* 11 (2020): e01610-20. PubMed: 32694143.
8. Young, B. E., et al. "Effects of a Major Deletion in the SARS-CoV-2 Genome on the Severity of Infection and the Inflammatory Response: An Observational Cohort Study." *Lancet* 396 (2020): 603-611. PubMed: 32822564.

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