

**Vector pLVX-EF1 $\alpha$ -IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Open Reading Frame 9b Gene**

**Catalog No. NR-52974**

**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 9b (orf9b) 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 9200 base pairs. NR-52974 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.

ORF9b is an alternative open reading frame within the nucleocapsid (N) gene of SARS-CoV-2. It has been shown in SARS-like coronaviruses to target mitochondria and significantly inhibit interferon I production.<sup>4,5,6</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-52974 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 9b Gene, NR-52974.”

**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. Krogan, N., Personal Communication.
2. Busby, M., et al. "Optimisation of a Multivalent Strep Tag for Protein Detection." *Biophys. Chem.* 152 (2010): 170-177. PubMed: 20970240.
3. Gordon, D. E., et al. "A SARS-CoV-2 Protein Interaction Map Reveals Targets for Drug Repurposing." *Nature* 583 (2020): 459-468. PubMed: 32353859.
4. Yoshimoto, F. K. "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.
5. Shi, C.-S., et al. "SARS-Coronavirus Open Reading Frame-9b Suppresses Innate Immunity by Targeting Mitochondria and the MAVS/TRAF3/TRAF6 Signalingosome." *J. Immunol.* 193 (2014): 3080-3089. PubMed: 25135833.
6. Jiang, H.-W., et al. "SARS-CoV-2 Orf9b Suppresses Type I Interferon Responses by Targeting TOM70." *Cell Mol. Immunol.* 17 (2020): 998-1000. PubMed: 32728199.

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