

Vector pLVX-EF1 α -IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Open Reading Frame 6 Gene

Catalog No. NR-52969

For research use only. Not for use in humans.

Contributor:

Nevan Krogan, Ph.D., Professor, Department of Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, California, USA

Manufacturer:

BEI Resources

Product Description:

Note: The vial label indicates this product contains a TST tag. This nomenclature refers to a 2X Strep tag.^{1,2} This product does not express the Twin-Strep-tag[®] that is commonly referred to as a TST tag.

The open reading frame 6 (orf6) 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.^{1,2,3} 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 α) 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 9100 base pairs. NR-52969 can be used for transient expression and lentivirus generation.¹ The complete plasmid sequence and map are provided on the BEI Resources webpage. The plasmid was produced in *E. coli* and extracted.

ORF6 is a SARS-CoV-2 accessory protein that is important for viral pathogenesis. ORF6 has been shown to interact with NSP8 during viral replication and inhibits type I interferon production and downstream signaling.^{4,5,6}

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 mammalian expression studies.

Packaging/Storage:

NR-52969 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 α -IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Open Reading Frame 6 Gene, NR-52969.”

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. 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. "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. Kumar, P., et al. "The Nonstructural Protein 8 (NSP8) of the SARS Coronavirus Interacts with its ORF6 Accessory Protein." Virology 366 (2007): 293-303. PubMed: 17532020.
6. Lei, X., et al. "Activation and Evasion of Type I Interferon Responses by SARS-CoV-2." Nat. Commun. 11 (2020): 3810. PubMed: 32733001.

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