

Vector pLVX-EF1 α -IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Open Reading Frame 3a Gene

Catalog No. NR-52965

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.^{1,2} This product does not express the Twin-Strep-tag[®] that is commonly referred to as a TST tag.

The open reading frame 3a (orf3a) 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 9740 base pairs. NR-52965 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.

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 β , resulting in apoptosis and cytokine storm promotion.^{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-52965 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 3a Gene, NR-52965.”

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. 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. 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.
6. 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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