

Vector pLVX-EF1 α -IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Non-Structural Protein 14 Gene

Catalog No. NR-52962

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 non-structural protein 14 (nsp14) 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 an N-terminal 2X Strep tag and cloned into the [pLVX-EF1 \$\alpha\$ -IRES-Puro](#) lentiviral expression plasmid.^{1,2} 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. NR-52962 can be used for transient expression and lentivirus generation.¹ The resulting size of the plasmid is approximately 10,490 base pairs. The complete plasmid sequence and map are provided on the BEI Resources webpage. The plasmid was produced in *E. coli* and extracted.

NSP14 is a multifunctional protein located in the ORF1b polyprotein. The amino-terminal domain is a 3'-5' exoribonuclease (ExoN) involved in a replicative mismatch repair system.^{3,4} The carboxy-terminal domain is a cap (guanine-N7) methyltransferase that, together with NSP10 and NSP16, generates mRNA 5'-terminal caps.^{4,5}

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-52962 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 Non-Structural Protein 14 Gene, NR-52962."

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/bmbl5/index.htm.

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References:

1. Krogan, N., Personal Communication.
2. Gordon, D. E., et al. "A SARS-CoV-2 Protein Interaction Map Reveals Targets for Drug Repurposing." *Nature* 583 (2020): 459-468. PubMed: 32353859.
3. Denison, M. R., et al. "Coronaviruses: An RNA Proofreading Machine Regulates Replication Fidelity and Diversity." *RNA Biol.* 8 (2011): 270-279. PubMed: 21593585.
4. Subissi, L., et al. "SARS-CoV ORF1b-encoded Nonstructural Proteins 12-16: Replicative Enzymes as Antiviral Targets." *Antiviral Res.* 101 (2014): 122-130. PubMed: 24269475.
5. Jin, X., et al. "Characterization of the Guanine-N7 Methyltransferase Activity of Coronavirus Nsp14 on Nucleotide GTP." *Virus Res.* 176 (2013): 45-52. PubMed: 23702198.

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