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SUPPORTING INFECTIOUS DISEASE RESEARCH

Vector pLVX-EF1α-IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Non-Structural Protein 7 Gene

Catalog No. NR-52955

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

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

The non-structural protein 7 (nsp7) 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-EF1α-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-52955 can be used for transient expression and lentivirus generation.¹ The resulting size of the plasmid is approximately 9170 base pairs. The complete plasmid sequence and map are provided on the BEI Resources webpage. The plasmid was produced in E. coli and extracted.

NSP7 is located in the ORF1ab polyprotein and is an accessory subunit in the RNA-dependent RNA polymerase (RdRp, also known as NSP12) complex and together with NSP8 may function as a general processivity factor for RdRp. The RdRp is a target of antiviral medications.^{3,4}

Material Provided:

Each vial contains plasmid DNA in TE buffer (10 mM Tris-HCI, 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. <u>Note</u>: The contents of the vial should be used to replicate the plasmid in *E. coli* prior to mammalian expression studies.

Packaging/Storage:

NR-52955 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-EF1α-IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Non-Structural Protein 7 Gene, NR-52955."

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. <u>Biosafety in Microbiological and Biomedical Laboratories</u>. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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References:

- 1. Krogan, N., Personal Communication.
- Gordon, D. E., et al. "A SARS-CoV-2 Protein Interaction Map Reveals Targets for Drug Repurposing." <u>Nature</u> 583 (2020): 459-468. PubMed: 32353859.
- Zhai, Y., et al. "Insights into SARS-CoV Transcription and Replication from the Structure of the Nsp7-Nsp8 Hexadecamer." <u>Nat. Struct. Mol. Biol.</u> 12 (2005): 980-986. PubMed: 16228002.
- Hillen, H. S., et al. "Structure of Replicating SARS-CoV-2 Polymerase." <u>Nature</u> 584 (2020): 154-156. PubMed: 32438371.

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