Product Information Sheet for NR-52953

Vector pLVX-EF1α-IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 3C-Like Protease Gene, C145A Mutant

Catalog No. NR-52953

For research use only. Not for use in humans.

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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 C145A mutant of the 3C-like protease [3CLpro; also referred to as non-structural protein 5 (nsp5); amino acids 3264 to 3569 of ORF1a; GenPept: QHO60603] 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.[1,2,3,4] 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 (EF1α) 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-52953 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.

3CLpro (also referred to as main protease, Mpro) is a cysteine protease that, together with the papain-like protease (PLpro), processes the viral polyproteins in preparation for viral replication. It also releases the main replicative functions of the virus, such as RNA-dependent RNA polymerase (RdRp) and helicase.[5,6,7]

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-52953 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 3C-Like Protease Gene, C145A Mutant, NR-52953."

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.

Disclaimers:
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References:

1. Krogan, N., Personal Communication.
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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. Kneller, D. W., et al. "Structural Plasticity of SARS-CoV-2 3CL M^{pro} Active Site Cavity Revealed by Room Temperature X-Ray Crystallography." Nat. Commun. 11 (2020): 3202. PubMed: 32581217.
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6. Lin, C. -W., et al. "Characterization of *Trans*- and *Cis*-Cleavage Activity of the SARS Coronavirus 3CLpro Protease: Basis for the *in vitro* Screening of Anti-SARS Drugs." FEBS Lett. 574 (2004): 131-137. PubMed: 15358553.
7. Zhang, L., et al. "Crystal Structure of SARS-CoV-2 Main Protease Provides a Basis for Design of Improved α-Ketoamide Inhibitors." Science 368 (2020): 409-412. PubMed: 32198291.
8. 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.

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