

**Vector pLVX-EF1 $\alpha$ -IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Non-Structural Protein 4 Gene**

Catalog No. NR-52951

**Product Description:**

Note: The vial label indicates this product contains a TST tag. This nomenclature refers to a 2X Strep tag. This product does not express the Twin-Strep-tag<sup>®</sup> that is commonly referred to as a TST tag. The non-structural protein 4 (nsp4) 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. 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 $\alpha$ ) 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 deposited plasmid was transformed into NEB<sup>®</sup> Stable Competent *E. coli* cells (New England Biolabs<sup>®</sup> C3040H), grown in Luria-Bertani broth with ampicillin (100  $\mu$ g per mL) for 1 day at 30°C in an aerobic atmosphere, extracted using a Plasmid *Plus* Maxi Kit (QIAGEN<sup>®</sup> 12963) and vialled in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70037527

Manufacturing Date: 14JUL2020

TEST	SPECIFICATIONS	RESULTS
<b>Next-Generation DNA Sequencing</b>	~ 10,420 base pairs	10,416 base pairs <sup>1,2</sup>
<b>Genotypic Analysis</b> Sequencing of nsp4 insert (~ 1500 base pairs)	100% sequence identity to depositor's sequence 2X Strep tag sequence confirmed	100% sequence identity to depositor's sequence <sup>3</sup> 2X Strep tag sequence confirmed <sup>4</sup>
<b>Antibiotic Resistance</b> Ampicillin (encoded by beta-lactamase gene <i>bla</i> ) <sup>5</sup> Puromycin (encoded by puromycin n-acetyltransferase gene <i>pac</i> )	<i>bla</i> sequence present <i>pac</i> sequence present	<i>bla</i> sequence present <i>pac</i> sequence present
<b>Concentration by PicoGreen<sup>®</sup> Measurement</b>	$\geq 2$ $\mu$ g/mL	0.1 $\mu$ g in 20 $\mu$ L per vial (2.7 $\mu$ g/mL)
<b>Amount per Vial</b>	Report results	0.1 $\mu$ g per vial
<b>OD<sub>260</sub>/OD<sub>280</sub> Ratio</b>	1.7 to 2.1	1.7
<b>Effective Bacterial Transformation</b> NEB <sup>®</sup> Stable Competent <i>E. coli</i>	$\geq 50$ colonies per ng	117 colonies per ng

<sup>1</sup>The sequence was assembled pre-vial using the predicted sequence as the reference sequence. The complete plasmid sequence and map are provided on the BEI Resources webpage.

<sup>2</sup>Sequencing produced two contigs that mapped to the reference sequence and are contiguous with one another, and no other contigs. The larger contig contains the nsp4 insert, antibiotic resistance genes, the IRES and the EF1 $\alpha$  promoter, whereas the small contig contains vector backbone. Only the larger contig sequence is reported on the BEI Resources webpage.

<sup>3</sup>The NR-52951 insert was codon optimized but is 100% identical with the NSP4 within the SARS-CoV-2, USA-WA1/2020 ORF1ab polyprotein (GenPept: QHO60603.1).

<sup>4</sup>This 2X Strep tag is defined by the sequence N-WSHPQFEKGGGSGGGSGGGWSHPQFEK-C. For more information, please see Busby, M., et al. "Optimisation of a Multivalent Strep Tag for Protein Detection." *Biophys. Chem.* 152 (2010): 170-177. PubMed: 20970240.

<sup>5</sup>The antibiotic ampicillin degrades quickly during growth. Bacterial stationary phase should be minimized during plasmid expansion to avoid plasmid loss and increased antibiotic concentrations may be necessary.

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