

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

**Catalog No. NR-52962**

**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 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. 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: 70037729**

**Manufacturing Date: 22JUL2020**

TEST	SPECIFICATIONS	RESULTS
<b>Next-Generation DNA Sequencing</b>	10,490 base pairs	10,491 base pairs <sup>1</sup>
<b>Genotypic Analysis</b> Sequencing of nsp14 insert (~ 1580 base pairs)	$\geq$ 99% sequence identity to depositor's sequence 2X Strep tag sequence confirmed	100% sequence identity to depositor's sequence <sup>2</sup> 2X Strep tag sequence confirmed <sup>3</sup>
<b>Antibiotic Resistance</b> Ampicillin (encoded by beta-lactamase gene <i>bla</i> ) <sup>4</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>Agarose Gel Electrophoresis</b> Digestion with <i>Eco</i> RI and <i>Bam</i> HI (pre-vial)	~ 2 kb and ~ 9 kb	~ 2 kb and ~ 9 kb (Figure 1)
<b>Concentration by PicoGreen<sup>®</sup> Measurement</b>	$\geq$ 2 $\mu$ g/mL	0.1 $\mu$ g in 20 $\mu$ L per vial (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 (pre-vial)</b>	1.7 to 2.1	1.9
<b>Effective Bacterial Transformation</b> NEB <sup>®</sup> Stable Competent <i>E. coli</i> cells	$\geq$ 50 colonies per ng	338 colonies per ng

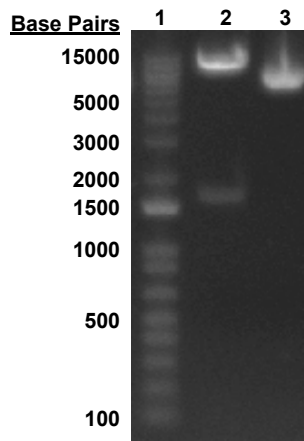
<sup>1</sup>The sequence was assembled pre-vial using the predicted sequence as the reference sequence. The complete plasmid sequence and map are provided on the BEI Resources webpage.

<sup>2</sup>The NR-52962 insert was codon optimized but is 100% identical with the SARS-CoV-2, USA-WA1/2020 ORF1ab polyprotein (GenPept QHO60603.1).

<sup>3</sup>This TST tag is a tandem Strep tag, defined by the sequence N-WSHPQFEKGGGSGGGSGGGSWHPQFEK-C. For more information, please see Gordon, D. E., et al. "A SARS-CoV-2 Protein Interaction Map Reveals Targets for Drug Repurposing." *Nature* 583 (2020): 459-468. PubMed: 32353859.

<sup>4</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.

Figure 1: Agarose Gel of Restriction Enzyme Digested and Undigested NR-52962



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder  
 Lane 2: NR-52962 digested  
 Lane 3: NR-52962 undigested

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Program Manager or designee, ATCC Federal Solutions

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