

**Vector pLVX-EF1 $\alpha$ -IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Open Reading Frame 9b Gene**

**Catalog No. NR-52974**

**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 open reading frame 9b (orf9b) 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: 70037927**

**Manufacturing Date: 05AUG2020**

TEST	SPECIFICATIONS	RESULTS
<b>Next-Generation DNA Sequencing</b>	~ 9200 base pairs	9204 base pairs <sup>1</sup>
<b>Genotypic Analysis</b> Sequencing of orf9b insert (~ 290 base pairs)	100% 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>Concentration by PicoGreen<sup>®</sup> Measurement</b>	$\geq 2$ $\mu$ g/mL	0.4 $\mu$ g in 40 $\mu$ L per vial (11 $\mu$ g/mL)
<b>Amount per Vial</b>	Report results	0.4 $\mu$ 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>	$\geq 50$ colonies per ng	138 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-52974 insert was codon optimized but is 100% identical with the SARS-CoV-2 OFR9b protein (UniProtKB: P0DTD2.1).

<sup>3</sup>This 2X Strep tag is defined by the sequence N-WSHPQFEKGGGSGGGSGGGSWHPQFEK-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>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.

/Heather Couch/

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16 OCT 2020

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