

Vector pLVX-EF1 α -IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Open Reading Frame 3a Gene

Catalog No. NR-52965

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[®] that is commonly referred to as a TST tag. The open reading frame 3a (orf3a) 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 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. The deposited plasmid was transformed into NEB[®] Stable Competent *E. coli* cells (New England Biolabs[®] C3040H), grown in Luria-Bertani broth with ampicillin (100 μ g per mL) for 1 day at 30°C in an aerobic atmosphere, extracted using a Plasmid *Plus* Maxi Kit (QIAGEN[®] 12963) and vialled in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70037733

Manufacturing Date: 22JUL2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 9740 base pairs	9738 base pairs ¹
Genotypic Analysis Sequencing of orf3a insert (825 base pairs)	≥ 99% sequence identity to depositor's sequence 2X Strep tag sequence confirmed	100% sequence identity to depositor's sequence ² 2X Strep tag sequence confirmed ³
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene <i>bla</i>) ⁴ 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
Agarose Gel Electrophoresis Digestion with <i>Bam</i> HI and <i>Eco</i> RI (pre-vial)	~ 9 kb and ~ 0.9 kb	~ 9 kb and ~ 0.9 kb (Figure 1)
Concentration by PicoGreen[®] Measurement	≥ 2 μ g/mL	0.4 μ g in 20 μ L per vial (19 μ g/mL)
Amount per Vial	Report results	0.4 μ g per vial
OD₂₆₀/OD₂₈₀ Ratio (pre-vial)	1.7 to 2.1	1.9
Effective Bacterial Transformation NEB [®] Stable Competent <i>E. coli</i>	≥ 50 colonies per ng	149 colonies per ng

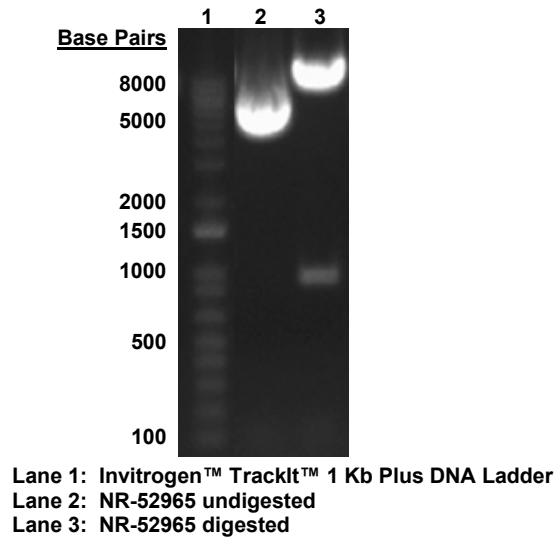
¹The sequence was assembled pre-vial using the predicted sequence as the reference sequence. The complete plasmid sequence and map are provided on the BEI Resources webpage.

²The NR-52965 insert was codon optimized but is 100% identical with the SARS-CoV-2 ORF3a protein (GenPept: QHO60595.1).

³This 2X Strep tag is defined by the sequence N-WSHQPFEKGGGSGGGSGGGSWSHQPFEK-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.

⁴The 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 Undigested and Restriction Enzyme Digested NR-52965



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