

Vector pLVX-EF1α-IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Open Reading Frame 3b Gene

Catalog No. NR-52966

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 3b (orf3b) 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 N-terminal 2X Strep tag and cloned into the [pLVX-EF1α-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 (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. The deposited plasmid was transformed into NEB® Stable Competent *E. coli* cells (New England Biolabs® C3040H), grown in Luria-Bertani broth with ampicillin (100 µg 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: 70037732

Manufacturing Date: 22JUL2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 9080 base pairs	9078 base pairs ¹
Genotypic Analysis Sequencing of orf3b insert (~ 170 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
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.1 µg in 20 µL per vial (7 µg/mL)
Amount per Vial	Report results	0.1 µg 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	325 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-52966 insert was codon optimized but is 100% identical with the SARS-CoV-2 ORF3b protein [see Kim, D.-K., et al. "A Comprehensive, Flexible Collection of SARS-CoV-2 Coding Regions." *G3 (Bethesda)* 10 (2020): 3399-3402. PubMed: 32763951].

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

/Heather Couch/

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16 OCT 2020

Program Manager or designee, ATCC Federal Solutions

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