Certificate of Analysis for NR-52953

Vector pLVX-EF1α-IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 3C-Like Protease Gene, C145A Mutant

Catalog No. NR-52953

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 C145A mutant of the 3C-like protease [3CLpro; also referred to as non-structural protein 5 (nsp5); amino acids 3264 to 3569 of ORF1a; GenPept: QHO60603] 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-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 vialed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70037529 **Manufacturing Date: 14JUL2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 9830 base pairs	9834 base pairs[1]
Genotypic Analysis Sequencing of 3CLpro insert (~ 920 base pairs)	≥ 99% sequence identity to depositor's sequence C145A mutation confirmed 2X Strep tag sequence confirmed	99.9% sequence identity to depositor's sequence[2] C145A mutation confirmed 2X Strep tag sequence confirmed[3]
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)[4] Puromycin (encoded by puromycin n-acetyltransferase gene *pac*)	*bla* sequence present *pac* sequence present	*bla* sequence present *pac* sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.3 µg in 20 µL per vial (14 µg/mL)
Amount per Vial	Report results	0.3 µg per vial
OD_{260}/OD_{280} Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation NEB® Stable Competent *E. coli* cells	≥ 50 colonies per ng	145 colonies per ng

[1]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.

[2]The NR-52953 insert was codon optimized but, other than the C145A mutation, is 100% identical with the 3CLpro of SARS-CoV-2, Wuhan-Hu-1 (GenPept: YP_009742612), which corresponds to amino acids 3264 to 3569 of the SARS-CoV-2, USA-WA1/2020 ORF1a protein (GenPept: QHO60603).

[3]This TST tag is a tandem Strep tag, defined by the sequence N-WSHPQFEKGGGSGGGSGGGSWSHPQFEK-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.

[4]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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