

Vector pCMV/R Containing the SARS-Related Coronavirus 2, Spike Glycoprotein Gene, Lineage B.1.1.7, Alpha Variant

Catalog No. NR-55304

This reagent is the tangible property of the U.S. Government.

Product Description:

NR-55304 expresses the full-length, Alpha variant spike (S) glycoprotein, and is intended for producing pseudotyped particles/pseudovirions. NR-55304 is not intended for recombinant protein expression. The vector for the S glycoprotein gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: [MN908947](#)) was designed by codon optimizing the full-length S sequence (residues 1 to 1273) for mammalian expression and introducing point mutations found in the B.1.1.7 lineage, resulting in a spike glycoprotein gene representative of the Alpha variant. The spike gene was subcloned into the pCMV/R mammalian expression vector (also referred to as VRC8400). The protein encoded by NR-55304 contains the following point mutations: H69del, V70del, Y144del, N501Y, A570D, D614G, P681H, T716I, S982A and D1118H. The kanamycin resistance gene, *aph*, provides transformant selection through kanamycin resistance in *Escherichia coli* (*E. coli*). NR-55304 is also referred to as VRC7596. The deposited plasmid was transformed into One Shot™ TOP10 *E. coli* (Invitrogen™ C404003), grown in Luria-Bertani broth with kanamycin (50 µg per mL) for 1 day at 37°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: 70046963

Manufacturing Date: 07SEP2021

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 8240 base pairs	8240 base pairs ¹
Genotypic Analysis Sequencing of S glycoprotein insert (~ 3820 base pairs) Lineage B.1.1.7 mutations	≥ 99% sequence identity to depositor's sequence Mutations present	100% sequence identity to depositor's sequence ² Mutations present
Antibiotic Resistance Kanamycin (encoded by kanamycin gene <i>aph</i>)	<i>aph</i> sequence present	<i>aph</i> sequence present
Concentration by Qubit Fluorometer®	≥ 2 µg per mL	0.2 µg in 20 µL per vial (12.4 µg per mL)
Amount per Vial	Report results	0.2 µg per vial
OD₂₆₀/OD₂₈₀ Ratio	1.7 to 2.1	2.0
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	94 colonies per ng

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

²The NR-55304 insert was codon optimized and B.1.1.7 mutations were introduced, but otherwise is 100% identical to the SARS-CoV-2, Wuhan-Hu-1 S protein (GenPept: YP_009724390.1).

/Heather Couch/

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