

**Vector pCMV/R Containing the SARS-Related Coronavirus 2, Spike Glycoprotein Gene, Lineage B.1.1.529, Omicron Variant**

**Catalog No. NR-56470**

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

**Product Description:**

**NR-56470 expresses the full-length, Omicron variant spike (S) glycoprotein, and is intended for producing pseudotyped particles/pseudovirions.<sup>1,2</sup> NR-56470 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.1.1.529 lineage, resulting in a spike glycoprotein gene representative of the Omicron variant. The spike gene was subcloned into the pCMV/R mammalian expression vector (also referred to as VRC8400). The protein encoded by NR-56470 contains the following lineage-defining point mutations: A67V, T95I, G339D, S371L, S373P, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H and N969K. The kanamycin resistance gene, *aph*, provides transformant selection through kanamycin resistance in *Escherichia coli* (*E. coli*). The resulting size of the plasmid is approximately 8240 base pairs. 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: 70049607**

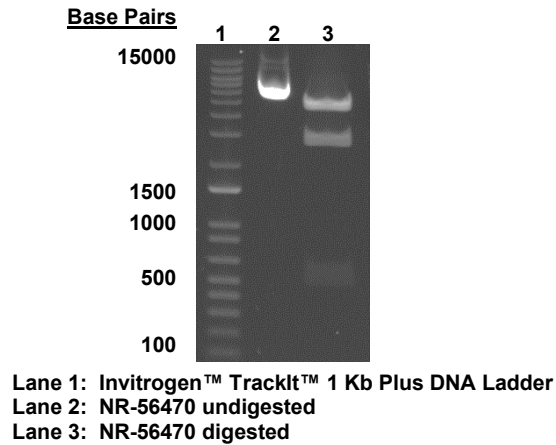
**Manufacturing Date: 28DEC2021**

TEST	SPECIFICATIONS	RESULTS
<b>Next-Generation DNA Sequencing</b>	~ 8240 base pairs	8240 base pairs <sup>1</sup>
<b>Genotypic Analysis</b> Sequencing of S glycoprotein insert (~ 3810 base pairs) Lineage B.1.1.529 mutations	≥ 99% sequence identity to depositor's sequence Mutations present	100% sequence identity to depositor's sequence <sup>2</sup> Mutations present
<b>Antibiotic Resistance</b> Kanamycin (encoded by <i>aph</i> )	<i>aph</i> sequence present	<i>aph</i> sequence present
<b>Agarose Gel Electrophoresis</b> Digestion with <i>SacI</i> (pre-vial)	~ 5 kb, ~ 3 kb and ~ 0.5 kb	~ 5 kb, ~ 3 kb and ~ 0.5 kb (Figure 1)
<b>Concentration by PicoGreen® Measurement</b>	≥ 2 µg/mL	0.3 µg in 20 µL per vial (14.5 µg/mL)
<b>Amount per Vial</b>	Report results	0.3 µg per vial
<b>OD<sub>260</sub>/OD<sub>280</sub> Ratio</b>	1.7 to 2.1	1.9
<b>Effective Bacterial Transformation</b> Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	155 colonies per ng

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

<sup>2</sup>The NR-56470 insert was codon optimized and B.1.1.529 mutations were introduced, but otherwise is 100% identical to the SARS-CoV-2, Wuhan-Hu-1 S protein (GenPept: YP\_009724390.1).

**Figure 1: Agarose Gel of Undigested and Restriction Enzyme Digested NR-56470**



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