b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-55305

Vector pCMV/R Containing the SARS-Related Coronavirus 2, Spike Glycoprotein Gene, Lineage B.1.351, Beta Variant

Catalog No. NR-55305

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

Product Description:

NR-55305 expresses the full-length, Beta variant spike (S) glycoprotein, and is intended for producing pseudotyped particles/pseudovirions. NR-55305 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.1.351 lineage, resulting in a spike glycoprotein gene representative of the Beta variant. The spike gene was subcloned into the pCMV/R mammalian expression vector (also referred to as VRC8400). The protein encoded by NR-55305 contains the following point mutations: L18F, D80A, D215G, LAL242-244del, R246I, K417N, E484K, N501Y, D614G and A701V. The kanamycin resistance gene, *aph*, provides transformant selection through kanamycin resistance in *Escherichia coli (E. coli)*. NR-55305 is also referred to as VRC7597. 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 vialed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70046965 **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.1.351 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 *aph*)	 *aph* sequence present	 *aph* sequence present
Concentration by Qubit Fluorometer®	≥ 2 µg per mL	1.7 µg in 90 µL per vial (19.4 µg per mL)
Amount per Vial	Report results	1.7 µg per vial
OD_{260}/OD_{280} Ratio	1.7 to 2.1	2.1
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	 ≥ 50 colonies per ng	 189 colonies per ng

[1]The 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.

[2]The NR-55305 insert was codon optimized and B.1.351 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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Program Manager or designee, ATCC Federal Solutions

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