

Certificate of Analysis for NR-53816

SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike-Pseudotyped Lentiviral Kit V2

Catalog No. NR-53816

Product Description:

Note: The NR-53742 vial label indicates this product has a 21 base pair deletion, but it is a 21 amino acid deletion. The severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: NC 045512) spike-pseudotyped lentiviral kit version 2 (NR-53816) is designed to generate pseudotyped lentiviral particles with the spike (S) glycoprotein gene, as well as luciferase (Luc2) and green fluorescent protein (GFP).

The deposited plasmids were transformed into One Shot™ TOP10 *E. coli* (Invitrogen™ C404003), grown in Luria-Bertani broth with ampicillin (50 to 100 µg/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).

Table 1: Lentiviral Kit Components

COMPONENT NUMBER	DESCRIPTION	LOT NUMBER	DATE OF MANUFACTURE
NR-53742	Viral entry protein encoding for S glycoprotein ΔCter	70038289	12AUG2020
NR-52516	Lentiviral Backbone encoding for Luc2 and ZsGreen	70062978	29APR2020 ¹
NR-52517	Helper plasmid encoding for Gag and Pol	70035478	29APR2020 ²
NR-52518	Helper plasmid encoding for Tat1b	70035480	29APR2020
NR-52519	Helper plasmid encoding for Rev1b	70035482	29APR2020

NR-52516 is a plasmid preparation derived from lot 70035475 that has been diluted 1:100 in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and dispensed on 24AUG2023 to produce lot 70062978.

Table 2: Viral Entry Protein (NR-53742)

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 8310 base pairs	8316 base pairs ¹
Genotypic Analysis Sequencing of S glycoprotein insert (~ 3760 base pairs)	≥ 99% sequence identity to depositor's sequence	100% sequence identity to depositor's sequence ²
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene <i>bla</i>) ³	bla sequence present	bla sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.7 µg in 30 µL per vial (22 µg/mL)
Amount per Vial	Report results	0.7 μg per vial
OD ₂₆₀ /OD ₂₈₀ Ratio (pre-vial)	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	248 colonies per ng

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.

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²NR-52517 is a plasmid preparation derived from lot 70035479 that has been diluted 1:100 in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and dispensed on 28AUG2023 to produce lot 70062979.

²The NR-53742 insert was codon optimized for mammalian expression, but otherwise is 100% identical to the SARS-CoV-2, Wuhan-Hu-1 S protein (GenPept: YP_009724390; residues 1-1252).

³The antibiotic ampicillin degrades quickly during growth. Bacterial stationary phase should be minimized during plasmid replication to avoid plasmid loss and increased antibiotic concentrations may be necessary.



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Table 3: Lentiviral Backbone (NR-52516)

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 9370 base pairs	9369 base pairs ¹
Genotypic Analysis Sequencing of Luc2 gene (~ 1650 base pairs) Sequencing of ZsGreen1 gene (~ 700 base pairs)	≥ 99% sequence identity to depositor's sequence ≥ 99% sequence identity to depositor's sequence	100% sequence identity to depositor's sequence 100% sequence identity to depositor's sequence
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene bla) ²	bla sequence present	bla sequence present
Concentration by Qubit Fluorometer® Measurement	≥ 2 µg/mL	0.36 μg in 20 μL/vial (18 μg/mL)
Amount per Vial	Report results	0.36 μg/vial
OD ₂₆₀ /OD ₂₈₀ Ratio (pre-vial)	1.7 to 2.1	1.8
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 Escherichia coli	≥ 50 colonies/ng	> 450 colonies/ng

¹The 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.

Table 4: Helper Plasmids (NR-52517 to NR-52519)

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	Report results	Consistent with depositor reported size ¹
Genotypic Analysis		
Sequencing of insertion	≥ 99% sequence identity to depositor's sequence	100% sequence identity to depositor's sequence
Antibiotic Resistance		
Ampicillin (encoded by beta-lactamase gene bla)2	bla sequence present	bla sequence present
Neomycin [NR-52519, encoded by aminoglycoside	aph(3')-II sequence present	aph(3')-II sequence present
3'-phosphotransferase gene aph(3')-II]		
Concentration ³		
NR-52517	≥ 2 µg/mL	0.26 μg in 20 μL per vial (13 μg/mL)
NR-52518	≥ 2 µg/mL	0.8 μg in 50 μL per vial (15 μg/mL)
NR-52519	≥ 2 µg/mL	0.9 μg in 70 μL per vial (13 μg/mL)
Amount per Vial		
NR-52517	Report results	0.26 μg
NR-52518	Report results	0.8 μg
NR-52519	Report results	0.9 µg
OD ₂₆₀ /OD ₂₈₀ Ratio (pre-vial)	1.7 to 2.1	1.7 to 2.1
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	> 450 colonies per ng

¹The 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.

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³The concentration for NR-52417 was determined by Qubit Fluorometer[®], and concentrations for NR-52418 and NR-52419 were determined by PicoGreen[®].



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/Sonia Bjorum Brower/ Sonia Bjorum Brower

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