

Certificate of Analysis for NR-52025

Vector VRC4235 Containing the Macaque Anti-Middle East Respiratory Syndrome Coronavirus Spike Monoclonal Antibody JC57-14 Kappa Chain Gene

Catalog No. NR-52025

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

Product Description:

NR-52025 is an expression vector containing a 702 base pair (VL+CL) insert that encodes a macaque anti-Middle East respiratory syndrome coronavirus (MERS-CoV) spike (S) monoclonal antibody JC57-14 kappa chain gene. The vector contains regulatory elements CMV enhancer/promoter, CMV IE splicing acceptor and HTLV-1 R region/splicing donor. Macaque Ig kappa chain leader is provided as the targeting sequence. The kanamycin resistance gene, *aph*, provides transformant selection through kanamycin resistance in *Escherichia coli* (*E. coli*). 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: 70047571 Manufacturing Date: 18OCT2021

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 5100 base pairs	5112 base pairs ¹
Genotypic Analysis Anti-MERS-CoV spike monoclonal antibody JC57-14 kappa chain gene (~ 702 base pairs)	≥ 99% sequence identity to depositor's sequence	99.9% sequence identity to depositor's sequence ²
Antibiotic Resistance Kanamycin (encoded by aph)	aph sequence present	aph sequence present
Concentration by Qubit Fluorometer®	≥ 2 µg/mL	0.4 μg in 20 μL/vial (19.9 μg/mL)
Amount per Vial	Report results	0.4 μg per vial
OD ₂₆₀ /OD ₂₈₀ Ratio	1.7 to 2.1	2.0
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	141 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.

/Sonia Bjorum Brower/ Sonia Bjorum Brower

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Technical Manager or designee, ATCC Federal Solutions

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²Comparison to the depositor's sequence indicates five SNPs, one of which is within the plasmid insert t1447c, resulting in a silent mutation.