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

Catalog No. NR-55304

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For research use only. Not for use in humans.

Contributor:

Barney Graham, Deputy Director and Chief, Vaccine Research Center, National Institutes of Health, Bethesda, Maryland, USA

Manufacturer:

BEI Resources

Product Description:

NR-55304 expresses the full-length, Alpha variant spike (S) glycoprotein, and is intended for producing pseudotyped particles/pseudovirions.[1,2] 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).[1,3,4] The protein encoded by NR-55304 contains the following point mutations: H69del, V70del, Y144del, N501Y, A570D, D614G, P681H, T716I, S982A and D1118H.[1] The kanamycin resistance gene, *aph*, provides transformant selection through kanamycin resistance in *Escherichia coli (E. coli)*.[1] NR-55304 is also referred to as VRC7596.[1] The resulting size of the plasmid is approximately 8240 base pairs. The complete plasmid sequence and map are provided on the BEI Resources webpage. The plasmid was produced in *E. coli* and extracted.

The S glycoprotein mediates viral binding to the host angiotensin converting enzyme 2 (ACE2). This protein forms a trimer, and when bound to a host receptor, allows fusion of the viral and cellular membranes. The S protein is a target for neutralizing antibodies.[5] The Alpha variant of SARS-CoV-2 includes multiple S glycoprotein mutations that were first identified in the United Kingdom, and the most studied is N501Y.[6] Structural modeling and mouse studies indicate N501Y increases S glycoprotein binding to ACE2, resulting in increased SARS-CoV-2 virulence.[7,8]

Material Provided:

Each vial contains plasmid DNA in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The DNA concentration and volume provided are shown on the Certificate of Analysis. The vial should be centrifuged prior to opening. Note: The contents of the vial should be used to replicate the plasmid in *E. coli* prior to mammalian expression.

Packaging/Storage:

NR-55304 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -20°C or colder immediately upon arrival. Freeze-thaw cycles should be minimized.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Vector pCMV/R Containing the SARS-Related Coronavirus 2, Spike Glycoprotein Gene, Lineage B.1.1.7, Alpha Variant, NR-55304."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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NR-55304 is claimed in U.S. Patent number 7,094,598 and the continuations, continuations-in-part, re-issues and foreign counterparts thereof.

References:

1. Graham, B., Personal Communication.
2. Millet, J. K., et al. "Production of Pseudotyped Particles to Study Highly Pathogenic Coronaviruses in a Biosafety Level 2 Setting." J. Vis. Exp. 145 (2019): doi: 10.3791/59010. PubMed: 30882796.
3. Wu, F., et al. "A New Coronavirus Associated with Human Respiratory Disease in China." Nature 579 (2020): 265-269. PubMed: 32015508.
4. Barouch, D. H., et al. "A Human T-Cell Leukemia Virus Type 1 Regulatory Element Enhances the Immunogenicity of Human Immunodeficiency Virus Type 1 DNA Vaccines in Mice and Nonhuman Primates." J. Virol. 79 (2005): 8828-8834. PubMed: 15994776.
5. Hulswit, R. J. G., C. A. M. de Haan and B.-J. Bosch. "Coronavirus Spike Protein and Tropism Changes." Adv. Virus Res. 96 (2016): 29-57. PubMed: 27712627.
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7. Gu, H., et al. "Adaptation of SARS-CoV-2 in BALB/c Mice for Testing Vaccine Efficacy." Science 369 (2020): 1603-1607. PubMed: 32732280.
8. Leung, K., et al. "Early Transmissibility Assessment of the N501Y Mutant Strains of SARS-CoV-2 in the United Kingdom, October to November 2020." Euro. Surveill. 26 (2021): pii 2002106. PubMed: 33413740.

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BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898