

SUPPORTING INFECTIOUS DISEASE RESEARCH

Product Information Sheet for NR-54006

Vector pCAGGS Containing the SARS-Related Coronavirus 2, Alpha Variant Spike Glycoprotein Receptor Binding Domain (RBD) Gene

Catalog No. NR-54006

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

For research use only. Not for use in humans.

Contributor:

Florian Krammer, Ph.D. and Fatima Amanat, Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, New York, USA, supported partially under government contract HHSN272201400008C, NIAID CEIRS program

Manufacturer:

BEI Resources

Product Description:

The vector for the receptor binding domain (RBD) of the spike (S) glycoprotein gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 MN908947) was designed by fusing the N-terminal S protein signal sequence to the spike RBD (amino acids 319 to 541) with a C-terminal hexa-histidine tag. 1,2 The sequence was codon optimized for mammalian expression, mutated to include the Alpha variant [also referred to as the United Kingdom (UK) variant; B.1.1.7 lineage] N501Y mutation and subcloned into the pCAGGS mammalian expression vector under the AG promoter.^{2,3} NR-54006 contains the beta-lactamase gene, bla, to provide transformant selection through ampicillin resistance in Escherichia coli (E. coli). The resulting size of the plasmid is approximately 5500 base pairs. The complete plasmid sequence and map are provided on the BEI Resources webpage. The plasmid was produced in E. coli and extracted.

Note: For a detailed protocol and list of related items, see https://labs.icahn.mssm.edu/krammerlab/covid-19/

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.⁴ 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.⁵ Structural modeling and mouse studies indicate N501Y increases S glycoprotein binding to ACE2, resulting in increased SARS-CoV-2 virulence.^{6,7}

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-54006 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 pCAGGS Containing the SARS-Related Coronavirus 2, Alpha Variant Spike Glycoprotein Receptor Binding Domain (RBD) Gene, NR-54006."

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.

Disclaimers:

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BEI Resources www.beiresources.org E-mail: contact@beiresources.org
Tel: 800-359-7370

Tel: 800-359-7370 Fax: 703-365-2898



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Registrants interested in commercializing this product must contact Mount Sinai for a license (Frenz, Christopher christopher.frenz@mssm.edu).

References:

- 1. Krammer, F. and F. Amanat, Personal Communication.
- Amanat, F., et al. "A Serological Assay to Detect SARS-CoV-2 Seroconversion in Humans." <u>Nat. Med.</u> 26 (2020): 1033-1036. PubMed: 32398876.
- Wu, F., et al. "A New Coronavirus Associated with Human Respiratory Disease in China." <u>Nature</u> 579 (2020): 265-269. PubMed: 32015508.
- Hulswit, R. J. G., C. A. M. de Haan and B.-J. Bosch. "Coronavirus Spike Protein and Tropism Changes." <u>Adv. Virus Res.</u> 96 (2016): 29-57. PubMed: 27712627.
- 5. <u>WHO</u>
- Gu, H., et al. "Adaptation of SARS-CoV-2 in BALB/c Mice for Testing Vaccine Efficacy." <u>Science</u> 369 (2020): 1603-1607. PubMed: 32732280.
- Leung, K., et al. "Early Transmissibility Assessment of the N501Y Mutant Strains of SARS-CoV-2 in the United Kingdom, October to November 2020." <u>Euro. Surveill.</u> 26 (2021): pii 2002106. PubMed: 33413740.

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E-mail: contact@beiresources.org Tel: 800-359-7370

Fax: 703-365-2898