Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, Kappa Variant with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells

Catalog No. NR-55495

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Contributor:

BEI Resources

Manufacturer:

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Product Description:

A recombinant form of the spike (S) glycoprotein from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Kappa variant (B.1.617.1 lineage) was produced in human embryonic kidney HEK293 cells and purified by immobilized metal affinity chromatography.[1,2,3,4] NR-55495 lacks the signal sequence and contains 1196 residues (ectodomain) of the SARS-CoV-2 S glycoprotein; the recombinant protein was stabilized by substitution at the furin S1/S2 cleavage site (RRAR→GSAS; residues 682 to 685) and KV→PP mutations (residues 986 and 987; wild type numbering), and includes a T4 foldon trimerization domain, HRV3C protease cleavage site and C-terminal octa-histidine tag fused to an AviTag™ BirA biotinylation acceptor sequence.[1,2,3] NR-55495 includes T95I, G142D, E154K, L452R, E484Q, D614G, P681R and Q1071H mutations in the S glycoprotein as compared to the SARS-CoV-2 reference sequence (GenPept: QHD43416).[1,5,6] The predicted protein sequence is shown in Figure 1.[1] NR-55495 has a theoretical molecular weight of 139,850 daltons. The crystal structure for trimeric S glycoprotein from SARS-CoV-2 has been solved at 3.46 Å resolution (PDB: 6VSB).[2]

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.[7] B.1.617.1 is one of several lineages and sublineages designated Kappa by the World Health Organization (WHO) and was first identified in India.[8] This lineage contains multiple mutations in the N-terminal domain (NTD) and the receptor-binding domain (RBD), such as L452R which has already been identified in other variants.[8,9,10] The L452R mutation has been shown to decrease sensitivity to neutralizing antibodies, increase viral infectivity and enhance viral replication capacity.[9,10,11,12]

Material Provided:

Each vial contains approximately 100 microliters of NR-55495 in 10 mM HEPES, pH 7, 150 mM NaCl and 2 mM ethylenediamine-tetraacetic acid (EDTA). The concentration, expressed as milligrams per milliliter, is shown on the Certificate of Analysis.

Packaging/Storage:

NR-55495 was packaged aseptically in cryovials. The product is provided on dry ice and should be stored at -20°C or colder immediately upon arrival. Storage at warmer temperatures is not recommended due to a low bioburden. Freeze-thaw cycles should be avoided.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, Kappa Variant with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells, NR-55495."

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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References:

1. Sather, D. N., Personal Communication.
2. Wrapp, D., et al. "Cryo-EM Structure of the 2019-nCoV Spike in the Prefusion Conformation." Science 367 (2020): 1260-1263. PubMed: 32075877.
3. Walls, A. C., et al. "Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein." Cell 181 (2020): 281-292. PubMed: 32155444.
4. Rambaut, A., et al. "A Dynamic Nomenclature Proposal for SARS-CoV-2 Lineages to Assist Genomic Epidemiology." Nat. Microbiol. 5 (2020): 1403-1407. PubMed: 32669681.
5. Wu, F., et al. "A New Coronavirus Associated with Human Respiratory Disease in China." Nature 579 (2020): 265-269. PubMed: 32015508.
6. West, A. P., Jr., et al. "Detection and Characterization of the SARS-CoV-2 Lineage B.1.526 in New York." bioRxiv (2021). doi: 10.1101/2021.02.14.431043. PubMed: 33907745.
7. 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.
8. WHO
9. Planas, D., et al. "Reduced Sensitivity of SARS-CoV-2 Variant Delta to Antibody Neutralization." Nature 5 596 (2021): 276-280. PubMed: 34237773.
10. Wilhelm, A., et al. "Antibody-Mediated Neutralization of Authentic SARS-CoV-2 B.1.617 Variants Harboring L452R and T478K/E484Q." Viruses 13 (2021): 1693. PubMed: 34578275.
11. Motozono, C., et al. "SARS-CoV-2 Spike L452R Variant Evades Cellular Immunity and Increases Infectivity." Cell Host Microbe 29 (2021): 1124-1136.e11. PubMed: 34171266.
12. Li, Q., et al. "The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity." Cell 182 (2020): 1284-1294.e9. PubMed: 32730807.

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Figure 1: Predicted Protein Sequence

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1    SQCVNLTTRT QLPPAYTNSF TRGVYYPDKV FRSSVLHSTQ DLFLPFFSNV
51   TWFHAIHVSG TNGTKRFDNP VLPFNDGVYF ASIEKSNIIR GWIFGTTLDS
101  KTQSLLIVNN ATNVVIKVCE FQFCNDPFLD VYYHKNNKSW MKSEFRVYSS
151  ANNCTFEYVS QPFLMDLEGK QGNFKNLREF VFKNIDGYFK IYSKHTPINL
201  VRDLPQGFSA LEPLVDLPIG INITRFQTLL ALHRSYLTPG DSSSGWTAGA
251  AAYYVGYLQP RTFLLKYNEN GTITDAVDCA LDPLSETKCT LKSFTVEKGI
301  YQTSNFRVQP TESIVRFPNI TNLCPFGEVF NATRFASVYA WNRKRISNCV
351  ADYSVLYNSA SFSTFKCYGV SPTKLNDLCF TNVYADSFVI RGDEVRQIAP
401  GQTGKIADYN YKLPDDFTGC VIAWNSNNLD SKVGGNYNYR YRLFRKSNLK
451  PFERDISTEI YQAGSTPCNG VQGFNCYFPL QSYGFQPTNG VGYQPYRVVV
501  LSFELLHAPA TVCGPKKSTN LVKNKCVNFN FNGLTGTGVL TESNKKFLPF
551  QQFGRDIADT TDAVRDPQTL EILDITPCSF GGVSVITPGT NTSNQVAVLY
601  QGVNCTEVPV AIHADQLTPT WRVYSTGSNV FQTRAGCLIG AEHVNNSYEC
651  DIPIGAGICA SYQTQTNSRG SASSVASQSI IAYTMSLGAE NSVAYSNNSI
701  AIPTNFTISV TTEILPVSMT KTSVDCTMYI CGDSTECSNL LLQYGSFCTQ
751  LNRALTGIAV EQDKNTQEVF AQVKQIYKTP PIKDFGGFNF SQILPDPSKP
801  SKRSFIEDLL FNKVTLADAG FIKQYGDCLG DIAARDLICA QKFNGLTVLP
851  PLLTDEMIAQ YTSALLAGTI TSGWTFGAGA ALQIPFAMQM AYRFNGIGVT
901  QNVLYENQKL IANQFNSAIG KIQDSLSSTA SALGKLQDVV NQNAQALNTL
951  VKQLSSNFGA ISSVLNDILS RLDPPEAEVQ IDRLITGRLQ SLQTYVTQQL
1001 IRAAEIRASA NLAATKMSEC VLGQSKRVDF CGKGYHLMSF PQSAPHGVVF
1051 LHVTYVPAHE KNFTTAPAIC HDGKAHFPRE GVFVSNGTHW FVTQRNFYEP
1101 QIITTDNTFV SGNCDVVIGI VNNTVYDPLQ PELDSFKEEL DKYFKNHTSP
1151 DVDLGDISGI NASVVNIQKE IDRLNEVAKN LNESLIDLQE LGKYEQGSGY
1201 IPEAPRDGQA YVRKDGEWVL LSTFLGRSLE VLFQGPGSHH HHHHHHGLND
1251 IFEAQKIEWH E
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Spike ectodomain – **Residues 1 to 1196** (represents WT amino acid residues 13 to 1208)
RRAR to GSAS substitution of S1/S2 cleavage site – Residues 670 to 673
KV to PP stabilizing mutations – Residues 974 and 975
T95I, G142D, E154K, L452R, E484Q, D614G, P681R and Q1071H mutations –
Residues 83, 130, 142, 440, 472, 602, 669 and 1059
T4 foldon trimerization domain – Residues 1199 to 1225
HRV3C protease cleavage site – Residues 1229 to 1236
Octa-histidine tag and AviTag™ – Residues 1239 to 1261

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