

Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, A.23.1 Lineage with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells

Catalog No. NR-55616

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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), A.23.1 lineage was produced in human embryonic kidney HEK293 cells and purified by immobilized metal affinity chromatography.^{1,2,3} NR-55616 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-55616 includes F157L, V367F, Q613H and P681R mutations in the S glycoprotein as compared to the SARS-CoV-2 reference sequence (GenPept: [QHD43416](#)).^{1,4,5} The predicted protein sequence is shown in Figure 1.¹ NR-55616 has a theoretical molecular weight of 139,800 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.⁵ The A.23.1 lineage emerged in mid-2020 in Uganda and includes the E484K and N501Y mutations in the S glycoprotein Receptor Binding Domain (RBD), which substantially compromise vaccine efficacy and antibody treatments.^{6,7}

Material Provided:

Each vial contains approximately 100 µL of NR-55616 in 10 mM HEPES, pH 7, 150 mM NaCl and 2 mM ethylenediamine-tetraacetic acid (EDTA). The concentration, expressed as mg per mL, is shown on the Certificate of Analysis.

Packaging/Storage:

NR-55616 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, A.23.1 Lineage with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells, NR-55616."

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. Wu, F., et al. "A New Coronavirus Associated with Human Respiratory Disease in China." Nature 579 (2020): 265-269. PubMed: 32015508.
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.
6. Bugembe, D. L., et al. "Emergence and Spread of a SARS-CoV-2 Lineage A Variant (A.23.1) with Altered Spike Protein in Uganda." Nat. Microbiol. 6 (2021): 1094-1101. PubMed: 34163035.
7. 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.

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Figure 1: Predicted Protein Sequence

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1   SQCVNLTRRT QLPPAYTNSF TRGVYYPDKV FRSSVLHSTQ DLFLPFFSNV
51  TWFHAIHVSG TNGTKRFDNP VLPFNDGVYF ASTEKSNIIR GWIFGTTLDS
101 KTQSLILVNN ATNVVIKVC E FQFCNDPFLG VYHKNNKSW MESELRVYSS
151 ANNCTFEYVS QPFLMDLEGK QGNFKNLREF VFKNIDGYFK IYSKHTPINL
201 VRDLPQGFSA LEPLVDLPIG INITRFQTL ALHRSYLTPG DSSSGWTAGA
251 AAYYVGYLQP RTFLLKYNE GTITDAVDCA LDPLSETKCT LKSFTVEKGI
301 YQTSNFRVQP TESIVRFPNI TNLCPFGEVF NATRFASVYA WNRKRISNCV
351 ADYSEFLYNSA SFSTFKCYGV SPTKLNLDL CF TNVYADSFVI RGDEVRQIAP
401 GQTGKIADYN YKLPDDFTGC VIAWNSNLD SKVGGNYNYL YRLFRSSNLK
451 PFERDISTEI YQAGSTPCNG VEGFNCYFPL QSYGFQPTNG VGYQPYRVVV
501 LSFELLHAPA TVCGPKKSTN LVKNKCVNFN FNGLTGTGVL TESNKKFLPF
551 QQFGRDIADT TDAVRDPQTL EILDITPCSF GGVSVITPGT NTSNQVAVLY
601 HDVNCTEVPV AIHADQLTPT WRVYSTGSNV FQTRAGCLIG AEHVNNSYEC
651 DIPIGAGICA SYQTQTNSRG SASSVASQSI IAYTMSLGAE NSVAYSNSI
701 AIPTNFTISV TTEILPVSMT KTSVDCTMYI CGDSTEC SNL 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 LHVTYVPAQE KNFTTAPAIC HDGKAHFPRE GVFVSNGTHW FVTQRNFYEP
1101 QIIITDNTFV 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

F157L, V367F, Q613H and P681R mutations –

Residues 145, 355, 601 and 669

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