

## Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, R.1 Lineage with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells

### Catalog No. NR-55632

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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), R.1 lineage was produced in human embryonic kidney HEK293 cells and purified by immobilized metal affinity chromatography.<sup>1,2,3</sup> NR-55632 lacks the signal sequence and contains 1196 residues (ectodomain) of the SARS-CoV-2 spike 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.<sup>1,2,3</sup> NR-55632 includes W152L, E484K, D614G and G769V mutations in the S glycoprotein as compared to the SARS-CoV-2 reference sequence (GenPept: [QHD43416](#)).<sup>1,4,5</sup> The predicted protein sequence is shown in Figure 1.<sup>1</sup> NR-55632 has a theoretical molecular weight of 139,600 daltons. The crystal structure for trimeric S glycoprotein from SARS-CoV-2 has been solved at 3.46 Å resolution (PDB: [6VSB](#)).<sup>2</sup>

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.<sup>6</sup> The R.1 lineage was predicted to have emerged around September 2020 and includes several key mutations of importance to the S glycoprotein: mutation W152L, which might reduce the effectiveness of neutralizing antibodies; mutation E484K, which has been identified in escape mutants from convalescent antisera, and is thought to play a role in the loss of antibody neutralizing activity; and mutation D614G, which is common to the current variants of interest and concern identified by the Centers for Disease Control and Prevention (CDC), was one of the first documented in the U.S. in the initial stages of the pandemic and demonstrates evidence of increasing virus transmissibility.<sup>7,8,9,10,11,12,13</sup>

#### Material Provided:

Each vial contains approximately 100 µL of NR-55632 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-55632 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, R.1 Lineage with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells, NR-55632."

#### 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](http://www.cdc.gov/biosafety/publications/bmbl5/index.htm).

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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 KTQSLLIVNN ATNVVIKVCE FQFCNDPFLG VYYHKNNKSL MESEFRVYSS
151 ANNCTFEYVS QPFLMDLEGK QGNFKNLREF VFKNIDGYFK IYSKHTPINL
201 VRDLPQGFSA LEPLVDLPIG INITRFQTLT ALHRSYLTPG DSSSGWTAGA
251 AAYYVGYLQP RTFLLKYNNEN GTITDAVDCA LDPLSETKCT LKSFTVEKGI
301 YQTSNFRVQP TESIVRFPNI TNLCPFGEVF NATRFASVYA WNRKRISNCV
351 ADYSVLYN SA SFSTFKCYGV SPTKLN DL CF TNVYADSFVI RGDEV RQIAP
401 GQTGKIADYN YKLPDDFTGC VIAWNSNNLD SKVGGNYNYL YRLFRKSNLK
451 PFERDISTEI YQAGSTPCNG VKGFNCYFPL QSYGFQPTNG VGYQPYRVVV
501 LSFELLHAPA TVCGPKKSTN LVKNKCVN FN FNGLTGTGVL TESNKKFLPF
551 QQFGRDIADT TDAVRDPQTL EILDITPCSF GGVSVITPGT NTSNQVAVLY
601 QGVNCTEVPV AIHADQLTPT WRVYSTGSNV FQTRAGCLIG AEHVNN SYEC
651 DIPIGAGICA SYQTQTNSPG SASSVASQSI IAYTMSLGAE NSVAYSNN SI
701 AIPTNFTISV TTEILPVSM T KTSVDCTMYI CGDSTEC SNL LLQYGSFCTQ
751 LNRALT V IAV EQDKNTQEVF AQVKQIYKTP PIKDFGGFNF SQILPDPSKP
801 SKRSFIEDLL FNKVTLADAG FIKQYGDCLG DIAARDLICA QKFNGLT VLP
851 PLLTDEMIAQ YTSALLAGTI TSGWTFGAGA ALQIPFAMQM AYRFNGIGVT
901 QNVLYENQKL IANQFNSAIG KIQDSLSTA SALGKLQDVV NQNAQALNTL
951 VKQLSSNFGA ISSVLNDILS RLDPPAEVQ IDRLITGRLQ SLQTYVTQQL
1001 IRAAEIRASA NLAATKMSEC VLGQSKRVDF CGKGYHLMSF PQSAPHGVVF
1051 LHVTYVPAQE KNFTTAPAIC HDGKAHFPRE GVFVSN GTHW FVTQRNFYEP
1101 QIITTDNTFV SGNCDDVIGI VNNTVYDPLQ PELDSFKEEL DKYFKNHTSP
1151 DVDLGDISGI NASVVNIQKE IDRLNEVAKN LNESLIDLQE LGKYEQ GSGY
1201 IPEAPRDGQA YVRKDGEWVL LSTFLGRSLE VLFQGP GSHH 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

W152L, E484K, D614G and G769V mutations –

**Residues 140, 472, 602 and 757**

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