Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, B.1.351 Lineage with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells

Catalog No. NR-55310

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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), South Africa variant (B.1.351 lineage) was produced in human embryonic kidney HEK293 cells and purified by immobilized metal affinity chromatography.[1,2,3] NR-55310 lacks the signal sequence and contains 1193 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.[1,2,3] NR-55310 is derived from the B.1.351 lineage of SARS-CoV-2, which includes L18F, D80A, D215G, del241-243, R246I, K417N, E484K, N501Y, D614G and A701V 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-55310 has a theoretical molecular weight of 139,380 daltons. The crystal structure for trimeric S glycoprotein from SARS-CoV-2, South Africa variant (B.1.351 lineage) has been solved at 3.65 Å resolution (PDB: 7LYK).[6]

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] New SARS-CoV-2 mutations in the S glycoprotein are currently under study, and the South African variant includes three mutations in the receptor binding domain (RBD) that may have functional significance, K417N, E484K and N501Y.[5] Structural modeling and mouse studies indicate N501Y increases S glycoprotein binding to ACE2, resulting in increased SARS-CoV-2 virulence.[8,9] In addition, the E484K mutation has been identified in escape mutants for convalescent antisera.[10]

Material Provided:
Each vial contains approximately 100 µL of NR-55310 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-55310 was packaged aseptically in cryovials. The product is provided on dry ice and should be stored at -20°C or colder immediately upon arrival. 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, B.1.351 Lineage with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells, NR-55310."

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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Figure 1: Predicted Protein Sequence

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1    SQCVNFTTRT QLPPAYTNSF TRGVYYPDKV FRSSVLHSTQ DLFLPFFSNV
51   TWFHAIHVSG TNGTKRFANP VLPFNDGVYF ASTEKSNIIR GWIFGTTLDS
101  KTQSLLIVNN ATNVVIKVCE FQFCNDPFLG VYYHKNNKSW MESEFRVYSS
151  ANNCTFEYVS QPFLMDLEGK QGNFKNLREF VFKNIDGYFK IYSKHTPINL
201  VRGLPQGFSA LEPLVDLPIG INITRFQTLH ISYLTPGDSS SGWTAGAAAY
251  YVGYLQPRTF LLKYNENGTI TDAVDCALDP LSETKCTLKS FTVEKGIYQT
301  SNFRVQPTES IVRFPNITNL CPFGEVFNAT RFASVYAWNR KRISNCVADY
351  SVLYNSASFS TFKCYGVSPT KLNDLCFTNV YADSFVIRGD EVRQIAPGQT
401  GNIADYNYKL PDDFTGCVIA WNSNNLDSKV GGNYNYLYRL FRKSNLKPFE
451  RDISTEIYQA GSTPCNGVKG FNCYFPLQSY GFQPTYGVGY QPYRVVVLSF
501  ELLHAPATVC GPKKSTNLVK NKCVNFNFNG LTGTGVLTES NKKFLPFQQF
551  GRDIADTTDA VRDPQTLEIL DITPCSFGGV SVITPGTNTS NQVAVLYQGV
601  NCTEVPVAIH ADQLTPTWRV YSTGSNVFQT RAGCLIGAEH VNNSYECDIP
651  IGAGICASYQ TQTNSPGSAS SVASQSIIAY TMSLGVENSV AYSNNSIAIP
701  TNFTISVTTE ILPVSMTKTS VDCTMYICGD STECSNLLLQ YGSFCTQLNR
751  ALTGIAVEQD KNTQEVFAQV KQIYKTPPIK DFGGFNFSQI LPDPSKPSKR
801  SFIEDLLFNK VTLADAGFIK QYGDCLGDIA ARDLICAQKF NGLTVLPPLL
851  TDEMIAQYTS ALLAGTITSG WTFGAGAALQ IPFAMQMAYR FNGIGVTQNV
901  LYENQKLIAN QFNSAIGKIQ DSLSSTASAL GKLQDVVNQN AQALNTLVKQ
951  LSSNFGAISS VLNDILSRLD PPEAEVQIDR LITGRLQSLQ TYVTQQLIRA
1001 AEIRASANLA ATKMSECVLG QSKRVDFCGK GYHLMSFPQS APHGVVFLHV
1051 TYVPAQEKNF TTAPAICHDG KAHFPREGVF VSNGTHWFVT QRNFYEPQII
1101 TTDNTFVSGN CDVVIGIVNN TVYDPLQPEL DSFKEELDKY FKNHTSPDVD
1151 LGDISGINAS VVNIQKEIDR LNEVAKNLNE SLIDLQELGK YEQGSGYIPE
1201 APRDGQAYVR KDGEWVLLST FLGRSLEVLF QGPGGSHHHH HHHHGLNDIF
1251 EAQKIEWHE
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Spike ectodomain – **Residues 1 to 1193** (represents WT amino acid residues 13 to 1208)
RRAR to GSAS substitution of S1/S2 cleavage site – Residues 667 to 670
KV to PP stabilizing mutations – Residues 971 and 972
L18F, D80A, D215G, R246I, K417N, E484K, N501Y, D614G and A701V Mutations – **Residues 6, 68, 203, 231, 402, 469, 486, 599 and 686**
T4 foldon trimerization domain – Residues 1196 to 1222
HRV3C protease cleavage site – Residues 1226 to 1233
Octa-histidine tag and AviTag™ – Residues 1237 to 1259

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