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Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, B.1.1.7 Lineage with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells

Catalog No. NR-55311

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Contributor:

BEI Resources

Manufacturer:

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Product Description:

A recombinant form of the spike (S) glycoprotein from severe respiratory syndrome-related coronavirus acute 2 (SARS-CoV-2), United Kingdom variant (UK; B.1.1.7 lineage) was produced in human embryonic kidney HEK293 cells and purified by immobilized metal affinity chromatography.^{1,2,3} NR-55311 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-55311 is derived from the B.1.1.7 lineage of SARS-CoV-2, which includes del69-70, del144, N501Y, A570D, D614G, P681H, T716I, S982A and D1118H mutations in the S glycoprotein as compared to the SARS-CoV-2 QHD43416).1,4,5 reference sequence (GenPept: The predicted protein sequence is shown in Figure 1.1 NR-55311 has a theoretical molecular weight of 139,500 daltons. The crystal structure for trimeric S glycoprotein from SARS-CoV-2, UK variant (B.1.1.7 lineage) has been solved at 3.22 Å resolution (PDB: 7LWS).5

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.⁶ The UK variants of SARS-CoV-2 include multiple mutations that were first identified in the United Kingdom, and the most studied is N501Y. Structural modeling and mouse studies indicate N501Y increases S glycoprotein binding to ACE2, resulting in increased SARS-CoV-2 virulence.^{7,8}

Material Provided:

Each vial contains approximately 100 μ L of NR-55311 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-55311 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.1.1.7 Lineage with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells, NR-55311."

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. <u>Biosafety in Microbiological and Biomedical Laboratories</u>. 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.
- Wrapp, D., et al. "Cryo-EM Structure of the 2019-nCoV Spike in the Prefusion Conformation." <u>Science</u> 367 (2020): 1260-1263. PubMed: 32075877.
- Walls, A. C., et al. "Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein." <u>Cell</u> 181 (2020): 281-292. PubMed: 32155444.
- Wu, F., et al. "A New Coronavirus Associated with Human Respiratory Disease in China." <u>Nature</u> 579 (2020): 265-269. PubMed: 32015508.
- Gobeil, S. M., et al. "Effect of Natural Mutations of SARS-CoV-2 on Spike Structure, Conformation and Antigenicity." <u>bioRxiv</u> (2021). doi: 10.1101/2021.03.11.435037. PubMed: 33758838.
- Hulswit, R. J. G., C. A. M. de Haan and B. -J. Bosch. "Coronavirus Spike Protein and Tropism Changes." <u>Adv.</u> <u>Virus Res.</u> 96 (2016): 29-57. PubMed: 27712627.
- 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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Figure 1: Predicted Protein Sequence

1	SQCVNLTTRT	QLPPAYTNSF	TRGVYYPDKV	FRSSVLHSTQ	DLFLPFFSNV
51	TWFHAISGTN	GTKRFDNPVL	PFNDGVYFAS	TEKSNIIRGW	IFGTTLDSKT
101	QSLLIVNNAT	NVVIKVCEFQ	FCNDPFLGVY	HKNNKSWMES	EFRVYSSANN
151	CTFEYVSQPF	LMDLEGKQGN	FKNLREFVFK	NIDGYFKIYS	KHTPINLVRD
201	LPQGFSALEP	LVDLPIGINI	TRFQTLLALH	RSYLTPGDSS	SGWTAGAAAY
251	YVGYLQPRTF	LLKYNENGTI	TDAVDCALDP	LSETKCTLKS	FTVEKGIYQT
301	SNFRVQPTES	IVRFPNITNL	CPFGEVFNAT	RFASVYAWNR	KRISNCVADY
351	SVLYNSASFS	TFKCYGVSPT	KLNDLCFTNV	YADSFVIRGD	EVRQIAPGQT
401	GKIADYNYKL	PDDFTGCVIA	WNSNNLDSKV	GGNYNYLYRL	FRKSNLKPFE
451	RDISTEIYQA	GSTPCNGVEG	FNCYFPLQSY	GFQPTYGVGY	QPYRVVVLSF
501	ELLHAPATVC	GPKKSTNLVK	NKCVNFNFNG	LTGTGVLTES	NKKFLPFQQF
551	GRDIDDTTDA	VRDPQTLEIL	DITPCSFGGV	SVITPGTNTS	NQVAVLYQGV
601	NCTEVPVAIH	ADQLTPTWRV	YSTGSNVFQT	RAGCLIGAEH	VNNSYECDIP
651	IGAGICASYQ	TQTNS <u>H</u> GSAS	SVASQSIIAY	TMSLGAENSV	AYSNNSIAIP
701	INFTISVTTE	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	VLNDILARLD	PPEAEVQIDR	LITGRLQSLQ	TYVTQQLIRA
1001	AEIRASANLA	ATKMSECVLG	QSKRVDFCGK	GYHLMSFPQS	APHGVVFLHV
1051	TYVPAQEKNF	TTAPAICHDG	KAHFPREGVF	VSNGTHWFVT	QRNFYEPQII
1101	TTHNTFVSGN	CDVVIGIVNN	TVYDPLQPEL	DSFKEELDKY	FKNHTSPDVD
1151	LGDISGINAS	VVNIQKEIDR	LNEVAKNLNE	SLIDLQELGK	YEQ GSGYIPE
1201	APRDGQAYVR	KDGEWVLLST	FLGRSLEVLF	QGPGGS <u>HHHH</u>	HHHHGLNDIF
1251	EAQKIEWHE				

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 N501Y, A570D, D614G, P681H, T716I, S982A and D1118H mutations – <u>Residues 486, 555, 599, 666, 701, 967 and 1103</u> T4 foldon trimerization domain – Residues 1196 to 1222 HRV3C protease cleavage site – Residues 1226 to 1233 Octa-histidine tag and AviTag[™] – <u>Residues 1237 to 1259</u>