Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, B.1.621 Lineage (Mu Variant) with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells

Catalog No. NR-55712

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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), B.1.621 lineage (Mu variant) was produced in human embryonic kidney HEK293 cells and purified by immobilized metal affinity chromatography.[1,2,3,4] NR-55712 lacks the signal sequence and contains 1197 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-55712 includes T95I, insert143T, Y144S, Y145N, R346K, E484K, N501Y, D614G, P681H and D950N 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-55712 has a theoretical molecular weight of 139,700 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.1.621 is one of several lineages and sublineages designated Mu by the World Health Organization (WHO) and was first identified in Columbia in January 2021.[8] This lineage contains several key mutations of importance to the S glycoprotein: the E484K mutation has been identified in escape mutants from convalescent antisera, and is thought to play a role in the loss of antibody neutralizing activity; N501Y increases S glycoprotein binding to ACE2, resulting in increased SARS-CoV-2 virulence; 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; and mutation P681H, part of the S1/S2 proteolytic cleavage site for furin proteases, results in increased cleavage efficiency and may alter antibody recognition sites.[9,10,11,12,13,14,15,16,17]

Material Provided:

Each vial contains approximately 100 µL of NR-55712 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-55712 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, B.1.621 Lineage (Mu Variant) with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells, NR-55712."

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    SQCVNLTTRT QLPPAYTNSF TRGVYYPDKV FRSSVLHSTQ DLFLPFFSNV
51   TWFHAIHVSG TNGTKRFDNP VLPFNDGVYF ASIEKSNIIR GWIFGTTLDS
101  KTQSLLIVNN ATNVVIKVCE FQFCNDPFLG VTSNHKNNKS WMESEFRVYS
151  SANNCTFEYV SQPFLMDLEG KQGNFKNLRE FVFKNIDGYF KIYSKHTPIN
201  LVRDLPQGFS ALEPLVDLPI GINITRFQTL LALHRSYLTP GDSSSGWTAG
251  AAAYYVGYLQ PRTFLLKYNE NGTITDAVDC ALDPLSETKC TLKSFTVEKG
301  IYQTSNFRVQ PTESIVRFPN ITNLCPFGEV FNATKFASVY AWNRKRISNC
351  VADYSVLYNS ASFSTFKCYG VSPTKLNDLC FTNVYADSFV IRGDEVRQIA
401  PGQTGKIADY NYKLPDDFTG CVIAWNSNNL DSKVGGNYNY LYRLFRKSNL
451  KPFERDISTE IYQAGSTPCN GVKGFNCYFP LQSYGFQPTY GVGYQPYRVV
501  VLSFELLHAP ATVCGPKKST NLVKNKCVNF NFNGLTGTGV LTESNKKFLP
551  FQQFGRDIAD TTDAVRDPQT LEILDITPCS FGGVSVITPG TNTSNQVAVL
601  YQGVNCTEVP VAIHADQLTP TWRVYSTGSN VFQTRAGCLI GAEHVNNSYE
651  CDIPIGAGIC ASYQTQTNSH GSASSVASQS IIAYTMSLGA ENSVAYSNNS
701  IAIPTNFTIS VTTEILPVSM TKTSVDCTMY ICGDSTECSN LLLQYGSFCT
751  QLNRALTGIA VEQDKNTQEV FAQVKQIYKT PPIKDFGGFN FSQILPDPSK
801  PSKRSFIEDL LFNKVTLADA GFIKQYGDCL GDIAARDLIC AQKFNGLTVL
851  PPLLTDEMIA QYTSALLAGT ITSGWTFGAG AALQIPFAMQ MAYRFNGIGV
901  TQNVLYENQK LIANQFNSAI GKIQDSLSST ASALGKLQNV VNQNAQALNT
951  LVKQLSSNFG AISSVLNDIL SRLDPPEAEV QIDRLITGRL QSLQTYVTQQ
1001 LIRAAEIRAS ANLAATKMSE CVLGQSKRVD FCGKGYHLMS FPQSAPHGVV
1051 FLHVTYVPAQ EKNFTTAPAI CHDGKAHFPR EGVFVSNGTH WFVTQRNFYE
1101 PQIITTDNTF VSGNCDVVIG IVNNTVYDPL QPELDSFKEE LDKYFKNHTS
1151 PDVDLGDISG INASVVNIQK EIDRLNEVAK NLNESLIDLQ ELGKYEQGSG
1201 YIPEAPRDGQ AYVRKDGEWV LLSTFLGRSL EVLFQGPGSH HHHHHHHGLN
1251 DIFEAQKIEW HE
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Spike ectodomain – **Residues 1 to 1197** (represents WT amino acid residues 13 to 1208)
RRAR to GSAS substitution of S1/S2 cleavage site – Residues 671 to 674
KV to PP stabilizing mutations – Residues 975 and 976
T95I, insert143T, Y144S, Y145N, R346K, E484K, N501Y, D614G, P681H and D950N mutations – **Residues 83, 132, 133, 134, 335, 473, 490, 603, 670 and 939**
T4 foldon trimerization domain – Residues 1200 to 1226
HRV3C protease cleavage site – Residues 1230 to 1237
Octa-histidine tag and AviTag™ – Residues 1240 to 1262

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