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

Catalog No. NR-55710

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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), AY.1 lineage (Delta variant) was produced in human embryonic kidney HEK293 cells and purified by immobilized metal affinity chromatography.[1,2,3,4] NR-55710 lacks the signal sequence and contains 1194 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-55710 includes T19R, T95I, G142D, E156G, delF157-R158, W258L, K417N, L452R, T478K, D614G, P681R 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-55710 has a theoretical molecular weight of 139,500 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] AY.1 is one of several lineages and sublineages designated Delta by the World Health Organization (WHO) and was first identified in India.[8] This lineage contains multiple mutations in the N-terminal domain (NTD) and the receptor-binding domain (RBD), such as L452R which has already been identified in other variants.[8,9] The L452R mutation has been shown to decrease sensitivity to neutralizing antibodies, increase viral infectivity and enhance viral replication capacity.[9,10,11]

Material Provided:

Each vial contains approximately 100 microliters of NR-55710 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-55710 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, AY.1 Lineage (Delta Variant) with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells, NR-55710."

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. 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.
5. Wu, F., et al. "A New Coronavirus Associated with Human Respiratory Disease in China." Nature 579 (2020): 265-269. PubMed: 32015508.
6. West, A. P., Jr., et al. "Detection and Characterization of the SARS-CoV-2 Lineage B.1.526 in New York." bioRxiv (2021). doi: 10.1101/2021.02.14.431043. PubMed: 33907745.
7. 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.
8. WHO
9. Planas, D., et al. "Reduced Sensitivity of SARS-CoV-2 Variant Delta to Antibody Neutralization." Nature 596 (2021): 276-280. PubMed: 34237773.
10. Motozono, C., et al. "SARS-CoV-2 Spike L452R Variant Evades Cellular Immunity and Increases Infectivity." Cell Host Microbe 29 (2021): 1124-1136.e11. PubMed: 34171266.
11. Li, Q., et al. "The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity." Cell 182 (2020): 1284-1294.e9. PubMed: 32730807.

Support Provided by NIAID

Figure 1: Predicted Protein Sequence

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1    SQCVNLRTRT QLPPAYTNSF TRGVYYPDKV FRSSVLHSTQ DLFLPFFSNV
51   TWFHAIHVSG TNGTKRFDNP VLPFNDGVYF ASIEKSNIIR GWIFGTTLDS
101  KTQSLLIVNN ATNVVIKVCE FQFCNDPFLD VYYHKNNKSW MESGVYSSAN
151  NCTFEYVSQP FLMDLEGKQG NFKNLREFVF KNIDGYFKIY SKHTPINLVR
201  DLPQGFSALE PLVDLPIGIN ITRFQTLLAL HRSYLTPGDS SSGLTAGAAA
251  YYVGYLQPRT FLLKYNENGT ITDAVDCALD PLSETKCTLK SFTVEKGIYQ
301  TSNFRVQPTE SIVRFPNITN LCPFGEVFNA TRFASVYAWN RKRISNCVAD
351  YSVLYNSASF STFKCYGVSP TKLNDLCFTN VYADSFVIRG DEVRQIAPGQ
401  TGNIADYNYK LPDDFTGCVI AWNSNNLDSK VGGNYNYRYR LFRKSNLKPF
451  ERDISTEIYQ AGSKPCNGVE GFNCYFPLQS YGFQPTNGVG YQPYRVVVLS
501  FELLHAPATV CGPKKSTNLV KNKCVNFNFN GLTGTGVLTE SNKKFLPFQQ
551  FGRDIADTTD AVRDPQTLEI LDITPCSFGG VSVITPGTNT SNQVAVLYQG
601  VNCTEVPVAI HADQLTPTWR VYSTGSNVFQ TRAGCLIGAE HVNNSYECDI
651  PIGAGICASY QTQTNSRGSA SSVASQSIIA YTMSLGAENS VAYSNNSIAI
701  PTNFTISVTT EILPVSMTKT SVDCTMYICG DSTECSNLLL QYGSFCTQLN
751  RALTGIAVEQ DKNTQEVFAQ VKQIYKTPPI KDFGGFNFSQ ILPDPSKPSK
801  RSFIEDLLFN KVTLADAGFI KQYGDCLGDI AARDLICAQK FNGLTVLPPL
851  LTDEMIAQYT SALLAGTITS GWTFGAGAAL QIPFAMQMAY RFNGIGVTQN
901  VLYENQKLIA NQFNSAIGKI QDSLSSTASA LGKLQNVVNQ NAQALNTLVK
951  QLSSNFGAIS SVLNDILSRL DPPEAEVQID RLITGRLQSL QTYVTQQLIR
1001 AAEIRASANL AATKMSECVL GQSKRVDFCG KGYHLMSFPQ SAPHGVVFLH
1051 VTYVPAQEKN FTTAPAICHD GKAHFPREGV FVSNGTHWFV TQRNFYEPQI
1101 ITTDNTFVSG NCDVVIGIVN NTVYDPLQPE LDSFKEELDK YFKNHTSPDV
1151 DLGDISGINA SVVNIQKEID RLNEVAKNLN ESLIDLQELG KYEQGSGYIP
1201 EAPRDGQAYV RKDGEWVLLS TFLGRSLEVL FQGPGSHHHH HHHHGLNDIF
1251 EAQKIEWHE
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Spike ectodomain – **Residues 1 to 1194** (represents WT amino acid residues 13 to 1208)
RRAR to GSAS substitution of S1/S2 cleavage site – Residues 668 to 671
KV to PP stabilizing mutations – Residues 972 and 973
T19R, T95I, G142D, E156G, W258L, K417N, L452R, T478K, D614G, P681R and D950N mutations –
Residues 7, 83, 130, 144, 244, 403, 438, 464, 600, 667 and 936
T4 foldon trimerization domain – Residues 1197 to 1223
HRV3C protease cleavage site – Residues 1227 to 1234
Octa-histidine tag and AviTag™ – Residues 1237 to 1259