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

Catalog No. NR-55307

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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), Brazil variant (P.1 lineage) was produced in human embryonic kidney HEK293 cells and purified by immobilized metal affinity chromatography.[1,2,3] NR-55307 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.[1,2,3] NR-55307 is derived from the P.1 lineage of SARS-CoV-2, which includes L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I and V1176F 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-55307 has a theoretical molecular weight of 139,850 daltons. The crystal structure for trimeric S glycoprotein from SARS-CoV-2, Brazil variant (B.1.1.28, an ancestor of P.1) has been solved at 3.22 Å resolution (PDB: 7LWW).[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] Structural modeling and mouse studies indicate N501Y increases S glycoprotein binding to ACE2, resulting in increased SARS-CoV-2 virulence.[7,8] In addition, the E484K mutation has been identified in escape mutants for convalescent antisera.[9]

Material Provided:

Each vial contains approximately 100 µL of NR-55307 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-55307 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, P.1 Lineage with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells, NR-55307."

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. Wu, F., et al. "A New Coronavirus Associated with Human Respiratory Disease in China." Nature 579 (2020): 265-269. PubMed: 32015508.
5. Gobeil, S. M., et al. "Effect of Natural Mutations of SARS-CoV-2 on Spike Structure, Conformation and Antigenicity." bioRxiv (2021). doi: 10.1101/2021.03.11.435037. PubMed: 33758838.
6. 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.
7. Gu, H., et al. "Adaptation of SARS-CoV-2 in BALB/c Mice for Testing Vaccine Efficacy." Science 369 (2020): 1603-1607. PubMed: 32732280.
8. Leung, K., et al. "Early Transmissibility Assessment of the N501Y Mutant Strains of SARS-CoV-2 in the United Kingdom, October to November 2020." Euro. Surveill. 26 (2021): pii 2002106. PubMed: 33413740.
9. Andreano, E., et al. "SARS-CoV-2 Escape *in vitro* from a Highly Neutralizing COVID-19 Convalescent Plasma." bioRxiv (2020). doi: 10.1101/2020.12.28.424451. PubMed: 33398278.

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Figure 1: Predicted Protein Sequence

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1    SQCVNFTNRT QLPSAYTNSF TRGVYYPDKV FRSSVLHSTQ DLFLPFFSNV
51   TWFHAIHVSG TNGTKRFDNP VLPFNDGVYF ASTEKSNIIR GWIFGTTLDS
101  KTQSLLIVNN ATNVVIKVCE FQFCNYPFLG VYYHKNNKSW MESEFRVYSS
151  ANNCTFEYVS QPFLMDLEGK QGNFKNLSEF VFKNIDGYFK IYSKHTPINL
201  VRDLPQGFSA LEPLVDLPIG INITRFQTLL ALHRSYLTPG DSSSGWTAGA
251  AAYYVGYLQP RTFLLKYNEN GTITDAVDCA LDPLSETKCT LKSFTVEKGI
301  YQTSNFRVQP TESIVRFPNI TNLCPFGEVF NATRFASVYA WNRKRISNCV
351  ADYSVLYNSA SFSTFKCYGV SPTKLNDLCF TNVYADSFVI RGDEVRQIAP
401  GQTGTIADYN YKLPDDFTGC VIAWNSNNLD SKVGGNYNYL YRLFRKSNLK
451  PFERDISTEI YQAGSTPCNG VKGFNCYFPL QSYGFQPTYG VGYQPYRVVV
501  LSFELLHAPA TVCGPKKSTN LVKNKCVNFN FNGLTGTGVL TESNKKFLPF
551  QQFGRDIADT TDAVRDPQTL EILDITPCSF GGVSVITPGT NTSNQVAVLY
601  QGVNCTEVPV AIHADQLTPT WRVYSTGSNV FQTRAGCLIG AEYVNNSYEC
651  DIPIGAGICA SYQTQTNSPG SASSVASQSI IAYTMSLGAE NSVAYSNNSI
701  AIPTNFTISV TTEILPVSMT KTSVDCTMYI CGDSTECSNL LLQYGSFCTQ
751  LNRALTGIAV EQDKNTQEVF AQVKQIYKTP PIKDFGGFNF SQILPDPSKP
801  SKRSFIEDLL FNKVTLADAG FIKQYGDCLG DIAARDLICA QKFNGLTVLP
851  PLLTDEMIAQ YTSALLAGTI TSGWTFGAGA ALQIPFAMQM AYRFNGIGVT
901  QNVLYENQKL IANQFNSAIG KIQDSLSSTA SALGKLQDVV NQNAQALNTL
951  VKQLSSNFGA ISSVLNDILS RLDPPEAEVQ IDRLITGRLQ SLQTYVTQQL
1001 IRAAEIRASA NLAAIKMSEC VLGQSKRVDF CGKGYHLMSF PQSAPHGVVF
1051 LHVTYVPAQE KNFTTAPAIC HDGKAHFPRE GVFVSNGTHW FVTQRNFYEP
1101 QIITTDNTFV SGNCDVVIGI VNNTVYDPLQ PELDSFKEEL DKYFKNHTSP
1151 DVDLGDISGI NASFVNIQKE IDRLNEVAKN LNESLIDLQE LGKYEQGSGY
1201 IPEAPRDGQA YVRKDGEWVL LSTFLGRSLE VLFQGPGGSH HHHHHHHGLN
1251 DIFEAQKIEW HE
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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
L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I and V1176F mutations – **Residues 6, 8, 14, 126, 178, 405, 472, 489, 602, 643, 1015 and 1164**
T4 foldon trimerization domain – Residues 1199 to 1225
HRV3C protease cleavage site – Residues 1229 to 1236
Octa-histidine tag and AviTag™ – Residues 1240 to 1262

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