

Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, BA.4 Lineage (Omicron Variant) with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells

Catalog No. NR-58646

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Contributor:

BEI Resources

Manufacturer:

D. Noah Sather, Associate Professor, Center for Global Infectious Disease Research, Seattle Children's Research Institute, Seattle, Washington, USA

Product Description:

A recombinant form of the spike (S) glycoprotein from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), BA.4 lineage (Omicron variant) was produced in human embryonic kidney HEK293 cells and purified by immobilized metal affinity and gel filtration chromatography.^{1,2,3,4} NR-58646 lacks the signal sequence and contains 1191 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 a C-terminal octa-histidine tag fused to an AviTag™ BirA biotinylation acceptor sequence.^{1,2,3} NR-58646 includes T19I, delL24-P26, A27S, delH69-V70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H and N969K mutations in the S glycoprotein, compared to the SARS-CoV-2 reference sequence (GenPept: [QHD43416](#)).^{1,5} The predicted protein sequence is shown in Figure 1.¹ NR-58646 has a theoretical molecular weight of 139,446 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.⁶ BA.4 is one of several lineages and sublineages designated Omicron by the World Health Organization (WHO).⁷ The spike protein of BA.4 is similar to BA.2, except for the addition of an amino acids 69 to 70 deletion, L452R, F486V and the wild type amino acid at Q493. Outside of the spike protein, BA.4 has additional mutations at ORF7b:L11F and N:P151S, a triple amino acid deletion in non-structural protein (NSP) NSP1:141-143 and a

nuc:G12160A mutation in NSP8.⁸ The L452R mutation is associated with increased evasion of cellular immunity and infectivity.⁹

Material Provided:

Each vial contains approximately 100 microliters of NR-58646 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-58646 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, BA.4 Lineage (Omicron Variant) with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells, NR-58646."

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.
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5. Wu, F., et al. "A New Coronavirus Associated with Human Respiratory Disease in China." Nature 579 (2020): 265-269. PubMed: 32015508.
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.
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8. Tegally, H., et al. "Emergence of SARS-CoV-2 Omicron Lineages BA.4 and BA.5 in South Africa." Nat. Med. (2022): doi:10.1038/s41591-022-01911-2. PubMed: 35760080.
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Figure 1: Predicted Protein Sequence

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1   SQCVNLITRT QSYTNSFTRG VYYPDKVFRS SVLHSTQDLF LPFFSNVTWF
51  HAISGTNGTK RFDNPVLPFN DGVYFASTEK SNIIRGWIFG TTLDSKTQSL
101 LIVNNATNVV IKVCEFQFCN DPFLDVYYHK NNKSWMESEF RVYSSANNCT
151 FEYVSQPFLM DLEGKQGNFK NLREFVFKNI DGYFKIYSKH TPINLGRDLP
201 QGFSALEPLV DLPIGINITR FQTLALHRS YLTPGDSSSG WTAGAAAYYV
251 GYLQPRFTLL KYNENGTITD AVDCALDPLS ETKCTLKSFT VEKGIYQTSN
301 FRVQPTESIV RFPNITNLCP FDEVFNATRF ASVYAWNRRK ISNCVADYSV
351 LYNFAPFFAF KCYGVSP TKL NDLCF TNVYA DSFVIRGNEV SQIAPGQTGN
401 IADYNYKLPD DFTGCVIAWN SNKLD SKVGG NYNYRYRLFR KSNLKPFRD
451 ISTEIYQAGN KPCNGVAGVN CYFPLQSYGF RPTYGVGHQP YRVVVL SFEL
501 LHAPATVCGP KKSTNLVKNK CVNFNFNGLT GTGVLTESNK KFLPFQQFGR
551 DIADTTDAVR DPQLEILDI TPCSF GGVSV ITPGTNTSNQ VAVLYQGVNC
601 TEVPVAIHAD QLTPTWRVYS TGSNVFQTRA GCLIGAEYVN NSYECDIPIG
651 AGICASYQTQ TKSHGSASSV ASQSIIAYTM SLGAENSVAY SNNSIAIPTN
701 FTISVTTEIL PVSMTKTSVD CTMYICGDST ECSNLLLQYG SFCTQLKRAL
751 TGIAVEQDKN TQEVFAQVKQ IYKTPPIKYF GGFNFSQILP DPSKPSKR SF
801 IEDLLFNKVT LADAGFIKQY GDCLGDIAAR DLICAQKFNG LTVLPPL LTD
851 EMIAQYTSAL LAGTITSGWT FGAGAALQIP FAMQMAYRFN GIGVTQNVLY
901 ENQKLIANQF NSAIGKIQDS LSSASALGK LQDVVNHN AQ ALNTLVKQLS
951 SKFGAISSVL NDILSRLDPP EAEVQIDRLI TGRLQSLQTY VTQQLIRAAE
1001 IRASANLAAT KMSECVLGQS KRVDFCGKGY HLMSFPQSAP HGVVFLHVTY
1051 VPAQEKNTT APAICHGKA HFPREGVFVS NGTHWFTQR NFYEPQIITT
1101 DNTFVSGNCD VVIGIVNNTV YDPLQPELDS FKEELDKYFK NHTSPDVDLG
1151 DISGINASVV NIQKEIDRLN EVAKNLNESL IDLQELGKYE QGSGYIPEAP
1201 RDGQAYVRKD GEWVLLSTFL GRSLEVL FQG PGSHHHHHHH HGLNDIFEAQ
1251 KIEWHE

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Spike ectodomain – **Residues 1 to 1191** (represents WT amino acid residues 13 to 1208)

RRAR to GSAS substitution of S1/S2 cleavage site – Residues 665 to 668

KV to PP stabilizing mutations – Residues 969 and 970

T19I, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H and N969K mutations –

Residues 7, 16, 125, 196, 322, 354, 356, 358, 359, 388, 391, 400, 423, 437, 460, 461,

467, 469, 481, 484, 488, 597, 638, 662, 664, 747, 779, 937 and 952

T4 foldon trimerization domain – Residues 1194 to 1220

HRV3C protease cleavage site – Residues 1224 to 1231

Octa-histidine tag and AviTag™ – Residues 1234 to 1256