

Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, B.1.526 Lineage with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells

Catalog No. NR-55438

This reagent is the tangible property of the U.S. Government.

For research use only. Not for use in humans.

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), New York variant (NY; B.1.526 lineage) was produced in human embryonic kidney HEK293 cells and purified by immobilized metal affinity chromatography.^{1,2,3} NR-55438 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-55438 is derived from the B.1.526 lineage of SARS-CoV-2, which includes L5F, T95I, D253G, E484K, D614G and A701V 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-55438 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.⁶ The B.1.526 lineage includes multiple mutations such as E484K, a mutation with rising dominance in NY infections in early 2021.^{7,8} 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.^{8,9}

Material Provided:

Each vial contains approximately 100 µL of NR-55438 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-55438 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.1.526 Lineage with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells, NR-55438."

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/bmb15/index.htm.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or

its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

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. 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.
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. Lasek-Nesselquist, E., et al. "The Localized Rise of a B.1.526 SARS-CoV-2 Variant Containing an E484K Mutation in New York State." medRxiv (2021). doi: 10.1101/2021.02.26.21251868.
8. Annavajhala, M. K., et al. "A Novel SARS-CoV-2 Variant of Concern, B.1.526, Identified in New York." medRxiv (2021). doi: 10.1101/2021.02.23.21252259. PubMed: 33655278.
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.

ATCC® is a trademark of the American Type Culture Collection.



Figure 1: Predicted Protein Sequence

```

1  SQCVNLTRRT QLPPAYTNSF TRGVYYPDKV FRSSVLHSTQ DLFLPFFSNV
51  TWFHAIHVSG TNGTKRFDNP VLPFNDGVYF ASIEKSNIIR GWIFGTTLDS
101  KTQSLLIVNN ATNVVIKVCE FQFCNDPFLG VYHKNNKSW MESEFRVYSS
151  ANNCTFEYVS QPFLMDLEGK QGNFKNREF VFKNIDGYFK IYSKHTPINL
201  VRDLPQGFSA LEPLVDLPIG INITRFQTL ALHRSYLTPG GSSSGWTAGA
251  AAYYVGYLQP RTFLLKYNEN GTITDAVDCA LDPLSETKCT LKSFTVEKGI
301  YQTSNFRVQP TESIVRFPNI TNLCPFGEVF NATRFASVYA WNRKRISNCV
351  ADYSVLYNSA SFSTFKCYGV SPTKLNLCF TNVYADSFVI RGDEVRQIAP
401  GQTGKIADYN YKLPDDFTGC VIAWNSNLD SKVGGNYNYL YRLFRRSNLK
451  PFERDISTEI YQAGSTPCNG VKGFNCYFPL QSYGFQPTNG VGYQPYRVVV
501  LSFELLHAPA TVCGPKKSTN LVKNKCVNFN FNGLTGTGVL TESNKKFLPF
551  QQFGRDIADT TDAVRDPQTL EILDITPCSF GGVSVITPGT NTSNQVAVLY
601  QGVNCTEVPV AIHADQLTPT WRVYSTGSNV FQTRAGCLIG AEHVNNSYEC
651  DIPIGAGICA SYQTQTNSPG SASSVASQSI IAYTMSLGVE NSVAYSNSI
701  AIPTNFTISV TTEILPVSMT KTSVDCMYI CGDSTECSNL LLQYGSFCTQ
751  LNRALTGIAV EQDKNTQEVF AQVKQIYKTP PIKDFGGFNF SQILPDPSKP
801  SKRSFIEDLL FNKVTLADAG FIKQYGDCLG DIAARDLICA QKFNGTLVLP
851  PLLTDEMIAQ YTSALLAGTI TSGWTFGAGA ALQIPFAMQM AYRFNGIGVT
901  QNVLYENQKL IANQFNSAIG KIQDSLSTA SALGKLQDVV NQNAQALNTL
951  VKQLSSNFGA ISSVLNDILS RLDPPEAEVQ IDRLITGRLQ SLQTYVTQQL
1001  IRAAEIRASA NLAATKMSEC VLGQSKRVDF CGKGYHLMSF PQSAPHGVVF
1051  LHVTYVPAQE KNETTAPAIC HDGKAHFPRE GVFVSNGLHW FVTQRNFYEP
1101  QIITDNTFV SGNCDVIGI VNNTVYDPLQ PELDSFKEEL DKYFKNHTSP
1151  DVDLGDISGI NASVVNIQKE IDRLNEVAKN LNESLIDLQE LGKYEQGSY
1201  IPEAPRDGQA YVRKDGEWVL LSTFLGRSLE VLFQGPGGSH HHHHHHGLN
1251  DIFEAQKIEW HE

```

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

T95I, D253G, E484K, D614G and A701V mutations –

Residues 83, 241, 472, 602 and 689

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