Product Information Sheet for NR-55421

Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, B.1.1.7 Variant (9 Mutations) with C-Terminal Histidine Tag, Recombinant from HEK293 Cells

Catalog No. NR-55421
ACROBiosystems Catalog No. SPN-C52H6

For research use only. Not for use in humans.

Contributor and Manufacturer:
ACROBiosystems, Newark, Delaware, USA

Product Description:
A recombinant form of the spike (S) glycoprotein from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), B.1.1.7 variant [United Kingdom (UK) variant; also known as 20B/501Y.V1 or VOC202012/01] was produced by transient transfection in human embryonic kidney HEK293 cells and purified by affinity chromatography.[1] NR-55421 lacks the signal sequence and contains 1195 residues (ectodomain) of the SARS-CoV-2 S glycoprotein; the recombinant protein was modified to remove the polybasic S1/S2 cleavage site (R683A and R685A), stabilized with multiple proline substitutions (F817P, A892P, A899P, A942P, K986P and V987P, wild type numbering) and includes a T4 fibritin trimerization motif and a poly-histidine tag at the C-terminus.[1,2,3] NR-55421 is a variant of SARS-CoV-2 which contains the HV69-70del, Y144del, N501Y, A570D, D614G, P681H, T716I, S982A and D1118H mutations in the S glycoprotein as compared to the SARS-CoV-2 reference sequence (GenPept: QHD43416).[1,4,5] The predicted protein residues are shown in Figure 1.[1] NR-55421 has a calculated molecular weight of 137.8 kilodaltons. The crystal structure for trimeric S glycoprotein from SARS-CoV-2, UK variant (B.1.1.7 lineage) has been solved at 3.22 Å resolution (PDB: 7LWS).[5] Representative gel filtration (SEC-MALS) and SDS-PAGE results are shown in Figures 2 and 3, respectively.[1]

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] New SARS-CoV-2 mutations in the S glycoprotein are currently under study, and the B.1.1.7 lineage includes the HV69-70del, Y144del, N501Y, A570D, D614G, P681H, T716I, S982A and D1118H mutations.[1,7] The B.1.1.7 lineage of SARS-CoV-2 includes multiple mutations that were first identified in the United Kingdom, and the most studied is N501Y. Structural modeling and mouse studies indicate N501Y increases S glycoprotein binding to ACE2, resulting in increased SARS-CoV-2 virulence.[8,9]

Material Provided:
Each vial contains approximately 50 µg of purified recombinant protein lyophilized in phosphate-buffered saline, pH 7.4 and 10% trehalose.

Packaging/Storage:
NR-55421 was packaged aseptically in glass vials. The product is provided lyophilized and should be placed in a closed, dry environment with desiccants and stored at -20ºC or colder immediately upon arrival. A frost-free freezer should be avoided, since changes in moisture and temperature may affect protein stability.

Functional Activity:
The biological activity of NR-55421 was measured by its binding ability in a functional ELISA (Figure 4), in which immobilized NR-55421 at 1 µg/mL (100 µL/well) can bind human ACE2 protein (Fc tag) (ACROBiosystems AC2-H5257); the linear range is 0.1 to 3 ng/mL.[1] Immobilized human ACE2 protein (Fc tag) (ACROBiosystems AC2-H5257) at 1 µg/mL (100 µL/well) can bind NR-55421; the linear range is 0.3 to 5 ng/mL (Figure 5).[1]

Reconstitution:
NR-55421 should be reconstituted with 83 µL sterile deionized water to a stock solution of 600 µg/mL. Add water at room temperature with occasional gentle mixing. Carrier protein [e.g. 0.1% (w/v) bovine serum albumin] must be included in the reconstitution buffer if the final protein concentration is lower than recommended or NR-55421 is aliquoted to less than 10 µg per vial. Note: Avoid vigorous shaking or vortexing.

Storage of Reconstituted Protein:
Reconstituted NR-55421 should be stored at -70°C or colder immediately and used within 3 months. Avoid repeated freeze-thaw cycles.

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.1.1.7 Variant (9 Mutations) with C-Terminal Histidine Tag, Recombinant from HEK293 Cells, NR-55421."

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 (BMBL). 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

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

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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. Chen, J., 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. Shen, L., et al. "Rapidly Emerging SARS-CoV-2 B.1.1.7 Sub-Lineage in the United States of America with Spike Protein D178H and Membrane Protein V70L Mutations." Emerg. Microbes Infect. (2021): *in press*. PubMed: 34125658.
8. Gu, H., et al. "Adaptation of SARS-CoV-2 in BALB/c Mice for Testing Vaccine Efficacy." Science 369 (2020): 1603-1607. PubMed: 32732280.
9. 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.

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

Support Provided by NIAID

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Figure 1: Predicted Target Protein Residues

```
1    VNLTTRTQLP PAYTNSFTRG VYYPDKVFRS SVLHSTQDLF LPFFSNVTWF
51   HAISGTNGTK RFDNPVLPFN DGVYFASTEK SNIIRGWIFG TTLDSKTQSL
101  LIVNNATNVV IKVCEFQFCN DPFLGVYHKN NKSWMESEFR VYSSANNCTF
151  EYVSQPFLMD LEGKQGNFKN LREFVFKNID GYFKIYSKHT PINLVRDLPQ
201  GFSALEPLVD LPIGINITRF QTLLALHRSY LTPGDSSSGW TAGAAAYYVG
251  YLQPRTFLLK YNENGTITDA VDCALDPLSE TKCTLKSFTV EKGIYQTSNF
301  RVQPTESIVR FPNITNLCPF GEVFNATRFA SVYAWNRKRI SNCVADYSVL
351  YNSASFSTFK CYGVSPTKLN DLCFTNVYAD SFVIRGDEVR QIAPGQTGKI
401  ADYNYKLPDD FTGCVIAWNS NNLDSKVGGN YNYLYRLFRK SNLKPFERDI
451  STEIYQAGST PCNGVEGFNC YFPLQSYGFQ PTYGVGYQPY RVVVLSFELL
501  HAPATVCGPK KSTNLVKNKC VNFNFNGLTG TGVLTESNKK FLPFQQFGRD
551  IDDTTDAVRD PQTLEILDIT PCSFGGVSVI TPGTNTSNQV AVLYQGVNCT
601  EVPVAIHADQ LTPTWRVYST GSNVFQTRAG CLIGAEHVNN SYECDIPIGA
651  GICASYQTQT NSHRAAASVA SQSIIAYTMS LGAENSVAYS NNSIAIPINF
701  TISVTTEILP VSMTKTSVDC TMYICGDSTE CSNLLLQYGS FCTQLNRALT
751  GIAVEQDKNT QEVFAQVKQI YKTPPIKDFG GFNFSQILPD PSKPSKRSPI
801  EDLLFNKVTL ADAGFIKQYG DCLGDIAARD LICAQKFNGL TVLPPLLTDE
851  MIAQYTSALL AGTITSGWTF GAGPALQIPF PMQMAYRFNG IGVTQNVLYE
901  NQKLIANQFN SAIGKIQDSL SSTPSALGKL QDVVNQNAQA LNTLVKQLSS
951  NFGAISSVLN DILARLDPPE AEVQIDRLIT GRLQSLQTYV TQQLIRAAEI
1001 RASANLAATK MSECVLGQSK RVDFCGKGYH LMSFPQSAPH GVVFLHVTYV
1051 PAQEKNFTTA PAICHDGKAH FPREGVFVSN GTHWFVTQRN FYEPQIITTH
1101 NTFVSGNCDV VIGIVNNTVY DPLQPELDSF KEELDKYFKN HTSPDVDLGD
1151 ISGINASVVN IQKEIDRLNE VAKNLNESLI DLQELGKYEQ YIKWP
```

Spike ectodomain – **Residues 1 to 1195** (represents WT amino acid residues 16 to 1213)
RRAR to RAAA substitution of S1/S2 cleavage site – Residues 664 to 667
KV to PP stabilizing mutations – Residues 968 and 969
N501Y, A570D, D614G, P681H, T716I, S982A and D1118H mutations –
Residues 483, 552, 596, 663, 697, 964 and 1100

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Figure 2: Representative SEC-MALS

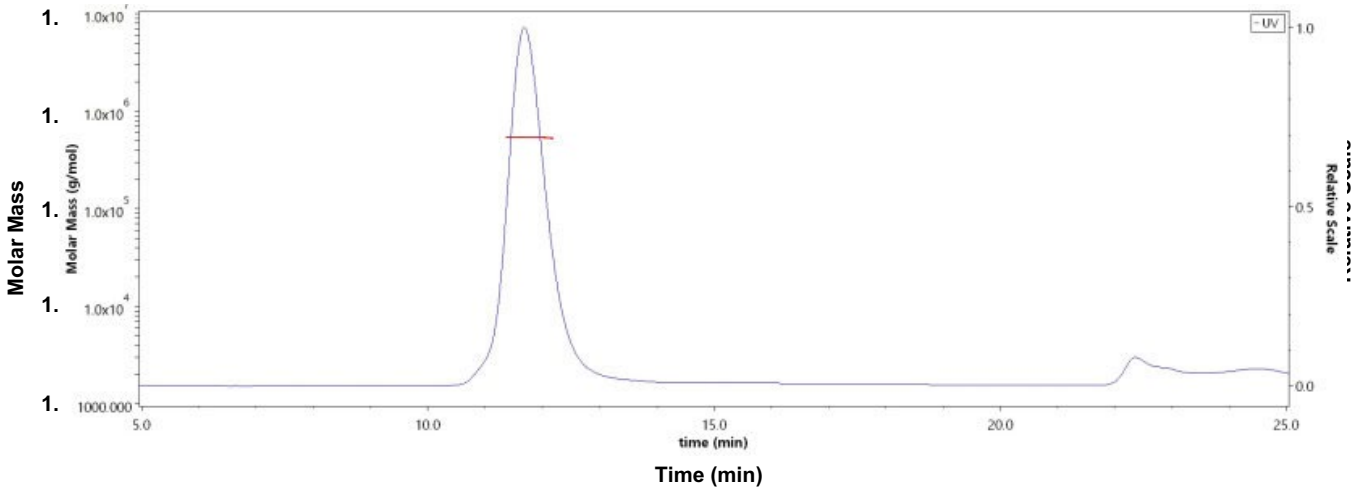
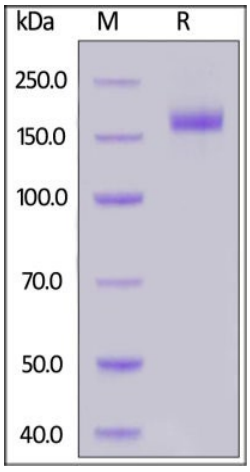
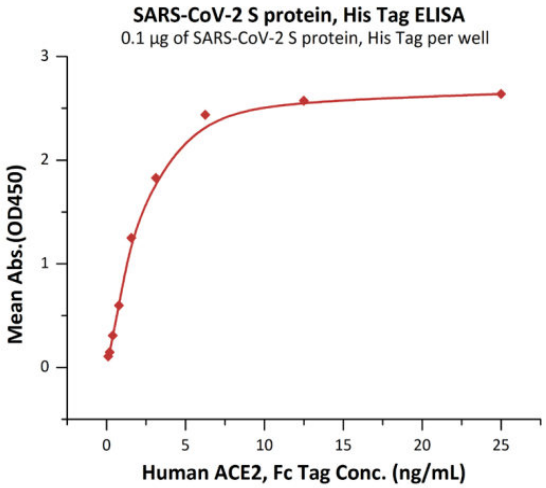


Figure 3: Representative SDS-PAGE



Lane 1: MW ladder
Lane 2: NR-55421

Figure 4: Representative ELISA



BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Figure 5: Representative ELISA