

Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, Wuhan-Hu-1 with C-Terminal Histidine and Twin-Strep® Tags, Recombinant from HEK293 Cells

Catalog No. NR-53589

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

For research use only. Not for human use.

Contributor and Manufacturer:

D. Noah Sather, Associate Professor, Peter Myler and Jason McLellan, 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), Wuhan-Hu-1 (GenPept: [QHD43416](#)) was produced by transient transfection of a previously published construct (VRC7471) into human embryonic kidney HEK293 cells and purified by immobilized metal affinity and gel filtration chromatography.^{1,2,3} NR-53589 lacks the native 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), and includes a T4 foldon trimerization domain, HRV3C protease cleavage site, and C-terminal octa-histidine and Twin-Strep® tags.^{1,4} The predicted protein sequence is shown in Figure 1. NR-53589 has a theoretical molecular weight of 140,700 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. The S protein is a target for neutralizing antibodies.⁵

Material Provided:

Each vial contains approximately 100 µL of NR-53589 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-53589 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, Wuhan-Hu-1 with C-Terminal Histidine and Twin-Strep® Tags, Recombinant from HEK293 Cells, NR-53589."

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. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; 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.

NR-53589 was produced from plasmid VRC7471 which is claimed in U.S. Provisional Patent Application number 16/344,774 and the continuations, continuations-in-part, re-issues and foreign counterparts thereof. The University of Texas-Austin, The Scripps Research Institute, Dartmouth College and the National Institutes of Health all have rights to this material.

References:

1. Sather, D. N., P. Myler and J. McLellan, 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. 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.

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



Figure 1 – Predicted Protein Sequence

```

1  CVNLTTRTQL PPAYTNSFTR GVYYDPKVFR SSVLHSTQDL FLPFFSNVTW
51  FHAIHVSQTN GTKRFDNPVL PFNDGVYFAS TEKSNIIRGW IFGTTLDSKT
101 QSLLIIVNNAT NVVIKVFCEQ FCNDPFLGVY YHKNNKSWME SEFRVYSSAN
151 NCTFEYVSQP FLMDLEGKQG NFKNLREFVF KNIDGYFKIY SKHTPINLVR
201 DLPQGFSALE PLVDLPIGIN ITRFQTLAL HRSYLTGDS SSGWTAGAAA
251 YYVGYLQPRF FLLKYNENGT ITDAVDCALD PLSETKCTLK SFTVEKGIYQ
301 TSNFRVQPTF SIVRFPNITN LCPFGEVFNA TRFASVYAWN RKRISNCVAD
351 YSVLYNSASF STFVKYGVSP TKLNDLCFTN VYADSFVIRG DEVRQIAPGQ
401 TGKIADYNYK LPDDFTGCVI AWNSNNLDSK VGGNYNYLYR LFRKSNLKPFL
451 ERDISTEIQY AGSTPCNGVE GFNCYFPLQS YGFQPTNGVG YQPYRVVVL
501 FELLHAPATV CGPKKSTNLV KNKCVNFNFN GLTGTGVLTE SNKKFLPFQ
551 FGRDIADTTD AVRDPQTLEI LDITPCSFSG VSVITPGTNT SNQVAVLYQD
601 VNCTEVPVAI HADQLTPTWR VYSTGSNVFQ TRAGCLIGAE HVNNSYECDI
651 PIGAGICASY QTQTNSPGSA SSVASQSIIA YTMSLGAENS VAYSNNIAI
701 PTNFTISVTT EILPVSMTKT SVDCTMYICG DSTECSNLLL QYGSFCTQLN
751 RALTGIAVEQ DKNTQEVFAQ VKQIYKTPPI KDFGGFNFSQ ILPDPSPKPSK
801 RSFIEDLLFN KVTLADAGFI KQYGDCLGDI AARDLICAQK ENGLTVLPL
851 LTDEMIAQYT SALLAGTITS GWTFGAGAAL QIPFAMQMAY RFNGIGVTQN
901 VLYENQKLIQ NQFNSAIGKI QDSLSTASA LGKLQDVVNQ NAQALNTLVK
951 QLSSNFGAIS SVLNDILSRL DPPEAEVQID RLITGRLOSL QTYVTQQLIR
1001 AAEIRASANL AATKMSECVL GQSKRVDFCG KGYHLMSFPQ SAPHGVVFLH
1051 VTYVPAQEKV FTTAPAICHD GKAHFPREGV FVSNNGTHWV TQRNFYEPQI
1101 ITTDNTFVSG NCDVVIGIVN NTVYDPLQPE LDSFKEELDK YFKNHTSPDV
1151 DLGDISGINA SVVNIQKEID RLNEVAKNLN ESLIDLQELG KYEQGSGYIP
1201 EAPRDGQAYV RKDGEWVLLS TFLGRSLEVL FQGPQHSHHH HHSASWSHPQ
1251 FEKGGGSGGG GSGGSAWSHP QFEK
    
```

Spike ectodomain – **Residues 1 to 1194** (represents WT amino acid residues 15 to 1208)
 RRAR to GSAS substitution of S1/S2 cleavage site – Residues 668 to 671
 KV to PP stabilizing mutations – Residues 972 and 973
 T4 foldon trimerization domain – Residues 1197 to 1223
 HRV3C protease cleavage site – Residues 1227 to 1234
 Octa-histidine tag – Residues 1236 to 1243
 Twin-Strep® tag – Residues 1246 to 1274