

SUPPORTING INFECTIOUS DISEASE RESEARCH

Product Information Sheet for NR-52308

Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, Wuhan-Hu-1 with C-Terminal Histidine Tag, Recombinant from Baculovirus

Catalog No. NR-52308

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

For research use only. Not for use in humans.

Contributor:

Florian Krammer, Ph.D., Fatima Amanat and Shirin Strohmeier, Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, New York, USA, supported partially under government contract HHSN272201400008C, NIAID CEIRS program

Manufacturer:

BEI Resources

Product Description:

A recombinant form of the spike (S) glycoprotein from severe respiratory syndrome-related coronavirus (SARS-CoV-2), Wuhan-Hu-1 (GenPept: QJE37812) was produced by transfection of purified plasmid in SF9 insect cells using a baculovirus expression system and purified by nickel affinity chromatography. 1,2,3,4 NR-52308 lacks the signal sequence and contains 1196 residues (ectodomain) of the SARS-CoV-2 spike glycoprotein; the recombinant protein was modified to remove the polybasic S1/S2 cleavage site (RRAR to A; residues 682 to 685), stabilized with a pair of mutations (K986P and V987P, wild type numbering; GenPept: QHD43416) and includes a thrombin cleavage site, T4 foldon trimerization domain and C-terminal hexa-histidine tag. 1,3 The predicted protein sequence is shown in Figure 1. NR-52308 has a theoretical molecular weight of 137,600 daltons. The crystal structure for trimeric S glycoprotein from SARS-CoV-2 has been solved at 3.20 angstrom resolution (PDB: 6VYB).2

Note: For a detailed protocol and list of related items, see https://labs.icahn.mssm.edu/krammerlab/covid-19/

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 of NR-52308 contains purified recombinant protein in phosphate buffered saline (PBS). The concentration and volume are shown on the Certificate of Analysis.

Note: The long-term stability of this preparation is not known. It is recommended that users confirm the activity of the product if not used within three months of receipt.

Packaging/Storage:

NR-52308 was packaged aseptically in cryovials. The product is provided on dry ice and should be stored at -60°C or colder immediately upon arrival. Freeze-thaw cycles should be avoided.

Functional Activity:

NR-52308 reacts with monoclonal anti-histidine tag in western blot analysis. NR-52308 is intended for western blot, ELISA and animal vaccination.^{1,3}

Citation:

Acknowledgment for publications should read "The following reagent was produced under HHSN272201400008C and obtained through BEI Resources, NIAID, NIH: Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, Wuhan-Hu-1 with C-Terminal Histidine Tag, Recombinant from Baculovirus, NR-52308."

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.

Disclaimers:

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References:

- Krammer, F., F. Amanat and S. Strohmeier, Personal Communication.
- Walls, A. C., et al. "Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein." <u>Cell</u> 181 (2020): 281-292. PubMed: 32155444.

- Amanat, F., et al. "A Serological Assay to Detect SARS-CoV-2 Seroconversion in Humans." <u>Nat. Med.</u> 26 (2020): 1033-1036. PubMed: 32398876.
- Wu, F., et al. "A New Coronavirus Associated with Human Respiratory Disease in China." <u>Nature</u> 579 (2020): 265-269. PubMed: 32015508.
- Hulswit, R. J. G., C. A. M. de Haan and B. -J. Bosch. "Coronavirus Spike Protein and Tropism Changes." <u>Adv. Virus Res.</u> 96 (2016): 29-57. PubMed: 27712627.

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Figure 1: Predicted Protein Sequence

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1
   CVNLTTRTQL PPAYTNSFTR GVYYPDKVFR SSVLHSTQDL FLPFFSNVTW
   FHAIHVSGTN GTKRFDNPVL PFNDGVYFAS TEKSNIIRGW IFGTTLDSKT
51
101 OSLLIVNNAT NVVIKVCEFO FCNDPFLGVY YHKNNKSWME SEFRVYSSAN
151 NCTFEYVSQP FLMDLEGKQG NFKNLREFVF KNIDGYFKIY SKHTPINLVR
201 DLPQGFSALE PLVDLPIGIN ITRFQTLLAL HRSYLTPGDS SSGWTAGAAA
251 YYVGYLQPRT FLLKYNENGT ITDAVDCALD PLSETKCTLK SFTVEKGIYQ
301 TSNFRVOPTE SIVRFPNITN LCPFGEVFNA TRFASVYAWN RKRISNCVAD
351 YSVLYNSASF STFKCYGVSP TKLNDLCFTN VYADSFVIRG DEVRQIAPGQ
401 TGKIADYNYK LPDDFTGCVI AWNSNNLDSK VGGNYNYLYR LFRKSNLKPF
451 ERDISTEIYO AGSTPCNGVE GFNCYFPLOS YGFOPTNGVG YOPYRVVVLS
501 FELLHAPATV CGPKKSTNLV KNKCVNFNFN GLTGTGVLTE SNKKFLPFQQ
551 FGRDIADTTD AVRDPQTLEI LDITPCSFGG VSVITPGTNT SNQVAVLYQD
601 VNCTEVPVAI HADQLTPTWR VYSTGSNVFQ TRAGCLIGAE HVNNSYECDI
651 PIGAGICASY OTOTNSPASV ASOSIIAYTM SLGAENSVAY SNNSIAIPTN
701 FTISVTTEIL PVSMTKTSVD CTMYICGDST ECSNLLLQYG SFCTQLNRAL
751 TGIAVEQDKN TQEVFAQVKQ IYKTPPIKDF GGFNFSQILP DPSKPSKRSF
801 IEDLLFNKVT LADAGFIKQY GDCLGDIAAR DLICAQKFNG LTVLPPLLTD
851 EMIAQYTSAL LAGTITSGWT FGAGAALQIP FAMQMAYRFN GIGVTQNVLY
901 ENQKLIANQF NSAIGKIQDS LSSTASALGK LQDVVNQNAQ ALNTLVKQLS
951 SNFGAISSVL NDILSRLDPP EAEVQIDRLI TGRLQSLQTY VTQQLIRAAE
1001 IRASANLAAT KMSECVLGQS KRVDFCGKGY HLMSFPQSAP HGVVFLHVTY
1051 VPAQEKNFTT APAICHDGKA HFPREGVFVS NGTHWFVTQR NFYEPQIITT
1101 DNTFVSGNCD VVIGIVNNTV YDPLQPELDS FKEELDKYFK NHTSPDVDLG
1151 DISGINASVV NIQKEIDRLN EVAKNLNESL IDLQELGKYE QYIKWPSGRL
1201 VPRGSPGSGY IPEAPRDGQA YVRKDGEWVL LSTFLGHHHH HH
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Spike ectodomain – **Residues 1 to 1196** (represents WT amino acid residues 15 to 1213)

RRAR to A substitution of S1/S2 cleavage site – Residue 671

KV to PP stabilizing mutations – Residues 969 and 970

Thrombin cleavage site – <u>Residues 1200 to 1205</u>

T4 foldon trimerization domain – Residues 1209 to 1235

Hexa-histidine tag – <u>Residues 1237 to 1242</u>

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