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SUPPORTING INFECTIOUS DISEASE RESEARCH

Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, D614G Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells

Catalog No. NR-55343 BPS Bioscience Catalog No. 100810

For research use only. Not for use in humans.

Contributor and Manufacturer:

BPS Bioscience, San Diego, California, USA

Product Description:

A recombinant form of the spike (S) glycoprotein from severe respiratory syndrome-related coronavirus acute 2 (SARS-CoV-2). D614G variant was produced in human embryonic kidney HEK293 cells and purified by affinity chromatography.¹ NR-55343 lacks the signal sequence (residues 1 to 15) and contains 1195 residues (ectodomain; S1 + S2) of the SARS-CoV-2 S 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) and includes a thrombin cleavage site, T4 foldon trimerization domain and C-terminal hexa-histidine tag.¹ NR-55343 is a variant of SARS-CoV-2 which contains the D614G mutation in the S glycoprotein as compared to the SARS-CoV-2 reference sequence (GenPept: <u>QHD43416</u>).^{1,2,3} The predicted protein sequence is shown in Figure 1.¹ NR-55343 has a theoretical molecular weight of 137,000 daltons. The crystal structure for trimeric S glycoprotein from the SARS-CoV-2 D614G variant has been solved at 3.46 Å resolution (PDB: 6XS6).3

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.⁴ The D614G mutation is common to the current variants of interest and concern identified by the Centers for Disease Control and Prevention (CDC). This mutation was one of the first documented in the USA in the initial stages of the pandemic after having initially circulated in Europe.⁵ Some evidence suggests that variants with the D614G mutation are more infectious than wild-type.⁶

Material Provided:

Each vial contains approximately 50 μ g of purified recombinant protein in 8 mM phosphate pH 7.4, 110 mM NaCl, 2.2 mM KCl and 20% glycerol. The concentration and volume are shown on the Certificate of Analysis.

Packaging/Storage:

NR-55343 was packaged aseptically in cryovials. The product is provided on dry ice and should be stored at -80°C immediately upon arrival. <u>Storage at warmer temperatures is</u> <u>not recommended due to a low bioburden</u>. 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, D614G Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells, NR-55343."

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. <u>Biosafety in Microbiological and Biomedical Laboratories</u>. 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:

- 1. Zhu, H., Personal Communication.
- Wu, F., et al. "A New Coronavirus Associated with Human Respiratory Disease in China." <u>Nature</u> 579 (2020): 265-269. PubMed: 32015508.

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- Hulswit, R. J. G., C. A. M. de Haan and B. -J. Bosch. "Coronavirus Spike Protein and Tropism Changes." <u>Adv.</u> <u>Virus Res.</u> 96 (2016): 29-57. PubMed: 27712627.
- Emary, K. R. W., et al. "Efficacy of ChAdOx1 nCoV-19 (AZD1222) Vaccine against SARS-CoV-2 Variant of Concern 202012/01 (B.1.1.7): An Exploratory Analysis of a Randomised Controlled Trial." <u>Lancet</u> 397 (2021): 1351-1362. PubMed: 33798499.
- Klumpp-Thomas, C., et al. "Effect of D614G Spike Variant on Immunoglobulin G, M, or A Spike Seroassay Performance." <u>J. Infect. Dis.</u> 223 (2021): 802-804. PubMed: 33257936.

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Figure 1: Predicted Protein Sequence

1	VNLTTRTQLP	PAYTNSFTRG	VYYPDKVFRS	SVLHSTQDLF	LPFFSNVTWF
51	HAIHVSGTNG	TKRFDNPVLP	FNDGVYFAST	EKSNIIRGWI	FGTTLDSKTQ
101	SLLIVNNATN	VVIKVCEFQF	CNDPFLGVYY	HKNNKSWMES	EFRVYSSANN
151	CTFEYVSQPF	LMDLEGKQGN	FKNLREFVFK	NIDGYFKIYS	KHTPINLVRD
201	LPQGFSALEP	LVDLPIGINI	TRFQTLLALH	RSYLTPGDSS	SGWTAGAAAY
251	YVGYLQPRTF	LLKYNENGTI	TDAVDCALDP	LSETKCTLKS	FTVEKGIYQT
301	SNFRVQPTES	IVRFPNITNL	CPFGEVFNAT	RFASVYAWNR	KRISNCVADY
351	SVLYNSASFS	TFKCYGVSPT	KLNDLCFTNV	YADSFVIRGD	EVRQIAPGQT
401	GKIADYNYKL	PDDFTGCVIA	WNSNNLDSKV	GGNYNYLYRL	FRKSNLKPFE
451	RDISTEIYQA	GSTPCNGVEG	FNCYFPLQSY	GFQPTNGVGY	QPYRVVVLSF
501	ELLHAPATVC	GPKKSTNLVK	NKCVNFNFNG	LTGTGVLTES	NKKFLPFQQF
551	GRDIADTTDA	VRDPQTLEIL	DITPCSFGGV	SVITPGTNTS	NQVAVLYQGV
601	NCTEVPVAIH	ADQLTPTWRV	YSTGSNVFQT	RAGCLIGAEH	VNNSYECDIP
651	IGAGICASYQ	TQTNSPASVA	SQSIIAYTMS	LGAENSVAYS	NNSIAIPTNF
701	TISVTTEILP	VSMTKTSVDC	TMYICGDSTE	CSNLLLQYGS	FCTQLNRALT
751	GIAVEQDKNT	QEVFAQVKQI	YKTPPIKDFG	GFNFSQILPD	PSKPSKRSFI
801	EDLLFNKVTL	ADAGFIKQYG	DCLGDIAARD	LICAQKFNGL	TVLPPLLTDE
851	MIAQYTSALL	AGTITSGWTF	GAGAALQIPF	AMQMAYRFNG	IGVTQNVLYE
901	NQKLIANQFN	SAIGKIQDSL	SSTASALGKL	QDVVNQNAQA	LNTLVKQLSS
951	NFGAISSVLN	DILSRLD <u>PP</u> E	AEVQIDRLIT	GRLQSLQTYV	TQQLIRAAEI
1001	RASANLAATK	MSECVLGQSK	RVDFCGKGYH	LMSFPQSAPH	GVVFLHVTYV
1051	PAQEKNFTTA	PAICHDGKAH	FPREGVFVSN	GTHWFVTQRN	FYEPQIITTD
1101	NTFVSGNCDV	VIGIVNNTVY	DPLQPELDSF	KEELDKYFKN	HTSPDVDLGD
1151	ISGINASVVN	IQKEIDRLNE	VAKNLNESLI	DLQELGKYEQ	YIKWP LVPRG
1201	<u>S</u> GYIPEAPRD	GQAYVRKDGE	WVLLSTFLGG	<u> GНННННН</u>	

Spike ectodomain – **Residues 1 to 1195** (represents WT amino acid residues 16 to 1213) RRAR to A substitution of S1/S2 cleavage site – <u>Residue 667</u> KV to PP stabilizing mutations – <u>Residues 968 and 969</u> D614G mutation – <u>Residues 598</u> Thrombin cleavage site – <u>Residues 1196 to 1201</u> T4 foldon trimerization domain – Residues 1202 to 1228 Hexa-histidine tag – <u>Residues 1232 to 1237</u>