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SUPPORTING INFECTIOUS DISEASE RESEARCH

Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, Delta Variant with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells

# Catalog No. NR-55614

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### **Contributor:**

**BEI Resources** 

#### Manufacturer:

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#### **Product Description:**

A recombinant form of the spike (S) glycoprotein from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Delta variant (B.1.617.2 lineage) was produced in human embryonic kidney HEK293 cells and purified by immobilized metal affinity chromatography.<sup>1,2,3,4</sup> NR-55614 lacks the 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; 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.<sup>1,2,3</sup> NR-55614 includes T19R, G142D, delE156-F157, R158G, L452R, T478K, D614G, P681R and D950N mutations in the S glycoprotein as compared to the SARS-CoV-2 reference sequence (GenPept: QHD43416).1,5,6 The predicted protein sequence is shown in Figure 1.1 NR-55614 has a theoretical molecular weight of 139,500 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.<sup>7</sup> B.1.617.2 is one of several lineages and sublineages designated Delta by the World Health Organization (WHO) and was first identified in India.<sup>8</sup> This lineage contains multiple mutations in the N-terminal domain (NTD) and the receptor-binding domain (RBD), such as L452R which has already been identified in other variants.<sup>8,9</sup> The L452R mutation has been shown to decrease sensitivity to neutralizing antibodies, increase viral infectivity and enhance viral replication capacity.<sup>9,10,11</sup>

# Material Provided:

Each vial contains approximately 100  $\mu L$  of NR-55614 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-55614 was packaged aseptically in cryovials. The product is provided on dry ice and should be stored at -20°C or colder immediately upon arrival. <u>Storage at warmer temperatures is</u> not recommended due to a low bioburden. 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, Delta Variant with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells, NR-55614."

### **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.

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#### **References:**

- 1. Sather, D. N., Personal Communication.
- Wrapp, D., et al. "Cryo-EM Structure of the 2019-nCoV Spike in the Prefusion Conformation." <u>Science</u> 367 (2020): 1260-1263. PubMed: 32075877.
- 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.
- Rambaut, A., et al. "A Dynamic Nomenclature Proposal for SARS-CoV-2 Lineages to Assist Genomic Epidemiology." <u>Nat. Microbiol.</u> 5 (2020): 1403-1407. PubMed: 32669681.
- Wu, F., et al. "A New Coronavirus Associated with Human Respiratory Disease in China." <u>Nature</u> 579 (2020): 265-269. PubMed: 32015508.
- West, A. P., Jr., et al. "Detection and Characterization of the SARS-CoV-2 Lineage B.1.526 in New York." <u>bioRxiv</u> (2021). doi: 10.1101/2021.02.14.431043. PubMed: 33907745.
- 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.
- 8. <u>WHO</u>
- Planas, D., et al. "Reduced Sensitivity of SARS-CoV-2 Variant Delta to Antibody Neutralization." <u>Nature</u> 5 596 (2021): 276-280. PubMed: 34237773.
- Motozono, C., et al. "SARS-CoV-2 Spike L452R Variant Evades Cellular Immunity and Increases Infectivity." <u>Cell</u> <u>Host Microbe</u> 29 (2021): 1124-1136.e11. PubMed: 34171266.
- 11. Li, Q., et al. "The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity." <u>Cell</u> 182 (2020): 1284-1294.e9. PubMed: 32730807.

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# Figure 1: Predicted Protein Sequence

1	SQCVNLRTRT	QLPPAYTNSF	TRGVYYPDKV	FRSSVLHSTQ	DLFLPFFSNV
51	TWFHAIHVSG	TNGTKRFDNP	VLPFNDGVYF	ASTEKSNIIR	GWIFGTTLDS
101	KTQSLLIVNN	ATNVVIKVCE	FQFCNDPFLD	VYYHKNNKSW	MES <u>G</u> VYSSAN
151	NCTFEYVSQP	FLMDLEGKQG	NFKNLREFVF	KNIDGYFKIY	SKHTPINLVR
201	DLPQGFSALE	PLVDLPIGIN	ITRFQTLLAL	HRSYLTPGDS	SSGWTAGAAA
251	YYVGYLQPRT	FLLKYNENGT	ITDAVDCALD	PLSETKCTLK	SFTVEKGIYQ
301	TSNFRVQPTE	SIVRFPNITN	LCPFGEVFNA	TRFASVYAWN	RKRISNCVAD
351	YSVLYNSASF	STFKCYGVSP	TKLNDLCFTN	VYADSFVIRG	DEVRQIAPGQ
401	TGKIADYNYK	LPDDFTGCVI	AWNSNNLDSK	VGGNYNYRYR	LFRKSNLKPF
451	ERDISTEIYQ	AGSKPCNGVE	GFNCYFPLQS	YGFQPTNGVG	YQPYRVVVLS
501	FELLHAPATV	CGPKKSTNLV	KNKCVNFNFN	GLTGTGVLTE	SNKKFLPFQQ
551	FGRDIADTTD	AVRDPQTLEI	LDITPCSFGG	VSVITPGTNT	SNQVAVLYQG
601	VNCTEVPVAI	HADQLTPTWR	VYSTGSNVFQ	TRAGCLIGAE	HVNNSYECDI
651	PIGAGICASY	<b>QTQTNSRGSA</b>	SSVASQSIIA	YTMSLGAENS	VAYSNNSIAI
701	PTNFTISVTT	EILPVSMTKT	SVDCTMYICG	DSTECSNLLL	QYGSFCTQLN
751	RALTGIAVEQ	DKNTQEVFAQ	VKQIYKTPPI	KDFGGFNFSQ	ILPDPSKPSK
801	RSFIEDLLFN	KVTLADAGFI	KQYGDCLGDI	AARDLICAQK	FNGLTVLPPL
851	LTDEMIAQYT	SALLAGTITS	GWTFGAGAAL	QIPFAMQMAY	RFNGIGVTQN
901	VLYENQKLIA	NQFNSAIGKI	QDSLSSTASA	LGKLQNVVNQ	NAQALNTLVK
951	QLSSNFGAIS	SVLNDILSRL	DPPEAEVQID	RLITGRLQSL	QTYVTQQLIR
1001	AAEIRASANL	AATKMSECVL	GQSKRVDFCG	KGYHLMSFPQ	SAPHGVVFLH
1051	VTYVPAQEKN	FTTAPAICHD	GKAHFPREGV	FVSNGTHWFV	TQRNFYEPQI
1101	ITTDNTFVSG	NCDVVIGIVN	NTVYDPLQPE	LDSFKEELDK	YFKNHTSPDV
1151	DLGDISGINA	SVVNIQKEID	RLNEVAKNLN	ESLIDLQELG	<b>Kyeq</b> gsgyip
1201	EAPRDGQAYV	RKDGEWVLLS	TFLGRSLEVL	FQGPGS <u>HHHH</u>	HHHHGLNDIF
1251	EAQKIEWHE				

Spike ectodomain – Residues 1 to 1194 (represents WT amino acid residues 13 to 1208) RRAR to GSAS substitution of S1/S2 cleavage site – Residues 668 to 671 KV to PP stabilizing mutations – Residues 972 and 973 T19R, G142D, R158G, L452R, T478K, D614G, P681R and D950N mutations – <u>Residues 7, 130, 144, 438, 464, 600, 667 and 936</u> T4 foldon trimerization domain – Residues 1197 to 1223 HRV3C protease cleavage site – Residues 1227 to 1234 Octa-histidine tag and AviTag<sup>™</sup> – <u>Residues 1237 to 1259</u>