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SUPPORTING INFECTIOUS DISEASE RESEARCH

Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, B.1.1.529 Lineage (Omicron Variant) with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells

## Catalog No. NR-56447

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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), B.1.1.529 lineage (Omicron variant) was produced in human embryonic kidney HEK293 cells and purified by immobilized metal affinity chromatography.<sup>1,2,3,4</sup> NR-56447 lacks the signal sequence and contains 1193 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  $KV \rightarrow PP$  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-56447 includes A67V, delH69-V70, T95I, G142D, delV143-Y145, delN211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K and L981F mutations in the S glycoprotein as compared to the SARS-CoV-2 reference sequence (GenPept: <u>QHD43416</u>).<sup>1,5</sup> The predicted protein sequence is shown in Figure 1.1 NR-56447 has a theoretical molecular weight of 139,900 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>6</sup> B.1.1.529 is one of several lineages and sublineages designated Omicron by the World Health Organization (WHO) and was first identified in South Africa, followed by multiple countries in November 2021.<sup>7</sup> This lineage contains multiple mutations in the receptor-binding domain (RBD) that have been identified in other variants, including K417N, N501Y and D614G.<sup>8,9,10</sup> The presence of D614G among variants has been shown to increase

transmissibility, with K417N, Q493R, N501Y and Y505H shown to be important residues mediating virus entry into host cells.<sup>9,11</sup> E484A has been shown to decrease neutralization by post-vaccination sera and some monoclonal antibody treatments.<sup>8,9,10</sup>

## Material Provided:

Each vial contains approximately 100 microliters of NR-56447 in 10 mM HEPES, pH 7, 150 mM NaCl and 2 mM ethylenediamine-tetraacetic acid (EDTA). The concentration, expressed as milligrams per milliliter, is shown on the Certificate of Analysis.

## Packaging/Storage:

NR-56447 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, B.1.1.529 Lineage (Omicron Variant) with C-Terminal Histidine and Avi Tags, Recombinant from HEK293 Cells, NR-56447."

### **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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# Figure 1: Predicted Protein Sequence

1	SQCVNLTTRT	QLPPAYTNSF	TRGVYYPDKV	FRSSVLHSTQ	DLFLPFFSNV
51	TWFHVISGTN	GTKRFDNPVL	PFNDGVYFAS	IEKSNIIRGW	IFGTTLDSKT
101	QSLLIVNNAT	NVVIKVCEFQ	FCNDPFLDHK	NNKSWMESEF	RVYSSANNCT
151	FEYVSQPFLM	DLEGKQGNFK	NLREFVFKNI	DGYFKIYSKH	TPIIVREPED
201	LPQGFSALEP	LVDLPIGINI	TRFQTLLALH	RSYLTPGDSS	SGWTAGAAAY
251	YVGYLQPRTF	LLKYNENGTI	TDAVDCALDP	LSETKCTLKS	FTVEKGIYQT
301	SNFRVQPTES	IVRFPNITNL	CPFDEVFNAT	RFASVYAWNR	KRISNCVADY
351	SVLYNLAPFF	TFKCYGVSPT	KLNDLCFTNV	YADSFVIRGD	EVRQIAPGQT
401	GNIADYNYKL	PDDFTGCVIA	WNSNKLDSKV	SGNYNYLYRL	FRKSNLKPFE
451	RDISTEIYQA	<b>GNKPCNGVAG</b>	FNCYFPLRSY	SFRPTYGVGH	QPYRVVVLSF
501	ELLHAPATVC	GPKKSTNLVK	NKCVNFNFNG	LKGTGVLTES	NKKFLPFQQF
551	GRDIADTTDA	VRDPQTLEIL	DITPCSFGGV	SVITPGTNTS	NQVAVLYQGV
601	NCTEVPVAIH	ADQLTPTWRV	YSTGSNVFQT	RAGCLIGAEY	VNNSYECDIP
651	IGAGICASYQ	TQTKSHGSAS	SVASQSIIAY	TMSLGAENSV	AYSNNSIAIP
701	TNFTISVTTE	ILPVSMTKTS	VDCTMYICGD	STECSNLLLQ	YGSFCTQLKR
751	ALTGIAVEQD	KNTQEVFAQV	KQIYKTPPIK	YFGGFNFSQI	LPDPSKPSKR
801	SFIEDLLFNK	VTLADAGFIK	QYGDCLGDIA	ARDLICAQKF	KGLTVLPPLL
851	TDEMIAQYTS	ALLAGTITSG	WTFGAGAALQ	IPFAMQMAYR	FNGIGVTQNV
901	LYENQKLIAN	QFNSAIGKIQ	DSLSSTASAL	GKLQDVVNHN	AQALNTLVKQ
951	LSSKFGAISS	VLNDI <u>F</u> SRLD	PPEAEVQIDR	LITGRLQSLQ	TYVTQQLIRA
1001	AEIRASANLA	ATKMSECVLG	QSKRVDFCGK	GYHLMSFPQS	APHGVVFLHV
1051	TYVPAQEKNF	TTAPAICHDG	KAHFPREGVF	VSNGTHWFVT	QRNFYEPQII
1101	TTDNTFVSGN	CDVVIGIVNN	TVYDPLQPEL	DSFKEELDKY	FKNHTSPDVD
1151	LGDISGINAS	VVNIQKEIDR	LNEVAKNLNE	SLIDLQELGK	<b>YEQ</b> GSGYIPE
1201	APRDGQAYVR	KDGEWVLLST	FLGRSLEVLF	QGPGS <u>HHHHH</u>	HHHGLNDIFE
1251	AQKIEWHE				

Spike ectodomain – **Residues 1 to 1193** (represents WT amino acid residues 13 to 1208) RRAR to GSAS substitution of S1/S2 cleavage site – Residues 667 to 670 KV to PP stabilizing mutations – Residues 971 and 972

A67V, T95I, G142D, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F –

## Residues 55, 81, 128, 194, 197-199, 324, 356, 358, 360, 402, 425, 431,

<u>462, 463, 469, 478, 481, 483, 486, 490, 532, 599, 640, 664, 666, 749, 781, 841, 939, 954, 966</u>

T4 foldon trimerization domain – Residues 1196 to 1222 HRV3C protease cleavage site – Residues 1226 to 1233 Octa-histidine tag and AviTag<sup>TM</sup> – <u>Residues 1236 to 1258</u>