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SUPPORTING INFECTIOUS DISEASE RESEARCH

# Spike Glycoprotein (Stabilized) from Human Coronavirus, HKU1 with C-Terminal Histidine and Avi Tags, Recombinant from HEK293F Cells

# Catalog No. NR-53713

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# For research use only. Not for human use.

## Contributor and Manufacturer:

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#### **Product Description:**

A recombinant form of the spike (S) glycoprotein from human coronavirus (HCoV), HKU1 (GenPept: AB<u>C70719</u>) was produced in human embryonic kidney HEK293F cells and purified by immobilized metal affinity and size exclusion chromatography.<sup>1,2</sup> NR-53713 lacks the signal sequence and contains 1264 residues (ectodomain) of the HCoV spike glycoprotein; the recombinant protein was stabilized by substitution at the furin S1/S2 cleavage site (RRKRR to GGSGS; residues 752 to 756) and with a pair of mutations (N1067P and L1068P, wild type numbering), and includes a thrombin cleavage site, T4 foldon trimerization domain and C-terminal hexa-histidine tag fused to an AviTag™ BirA biotinylation acceptor sequence.1,2 The predicted protein sequence is shown in Figure 1.<sup>1</sup> NR-53713 has a theoretical molecular weight of 147,070 daltons. The crystal structure for trimeric S glycoprotein from HCoV, HKU1 has been solved at 4.04 Å resolution (PDB: 5108).3

#### **Material Provided:**

Each vial contains approximately 50  $\mu$ L of NR-53713 in phosphate buffered saline (PBS; pH ~ 7). The concentration, expressed as mg per mL, is shown on the Certificate of Analysis.

### Packaging/Storage:

NR-53713 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 Human Coronavirus, HKU1 with C-Terminal Histidine and Avi Tags, Recombinant from HEK293F Cells, NR-53713."

#### **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</u> <u>Microbiological and Biomedical Laboratories</u>. 5th ed. Washington, DC: U.S. Government Printing Office, 2009.

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# References:

- 1. Strong, R. K., Personal Communication.
- Woo, P. C., et al. "Comparative Analysis of 22 Coronavirus HKU1 Genomes Reveals a Novel Genotype and Evidence of Natural Recombination in Coronavirus HKU1." <u>J. Virol.</u> 80 (2006): 7136-7145. PubMed: 16809319.
- Kirchdoerfer, R. N., et al. "Pre-Fusion Structure of a Human Coronavirus Spike Protein. Version 2." <u>Nature</u> 531 (2016): 118-121. PubMed: 26935699.

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- Woo, P. C., et al. "Characterization and Complete Genome Sequence of a Novel Coronavirus, Coronavirus HKU1, from Patients with Pneumonia." <u>J. Virol.</u> 79 (2005): 884-895. PubMed: 15613317.
- 5. Llanes, A., et. al. "Betacoronavirus Genomes: How Genomic Information has been Used to Deal with Past

Outbreaks and the COVID-19 Pandemic." <u>Int. J. Mol. Sci.</u> 21 (2020): 4546. PubMed: 32604724.

ATCC<sup>®</sup> is a trademark of the American Type Culture Collection.



### Figure 1 – Predicted Protein Sequence

1	VIGDFNCTNS	FINDYNKTIP	RISEDVVDVS	LGLGTYYVLN	RVYLNTTLLF
51	TGYFPKSGAN	FRDLALKGSI	YLSTLWYKPP	FLSDFNNGIF	SKVKNTKLYV
101	NNTLYSEFST	IVIGSVFVNT	SYTIVVQPHN	GILEITACQY	TMCEYPHTVC
151	KSKGSIRNES	WHIDSSEPLC	LFKKNFTYNV	SADWLYFHFY	QERGVFYAYY
201	ADVGMPTTFL	FSLYLGTILS	HYYVMPLTCN	AISSNTDNET	LEYWVTPLSR
251	RQYLLNFDEH	GVITNAVDCS	SSFLSEIQCK	TQSFAPNTGV	YDLSGFTVKP
301	VATVYRRIPN	LPDCDIDNWL	NNVSVPSPLN	WERRIFSNCN	FNLSTLLRLV
351	HVDSFSCNNL	DKSKIFGSCF	NSITVDKFAI	PNRRRDDLQL	GSSGFLQSSN
401	YKIDISSSSC	QLYYSLPLVN	VTINNFNPSS	WNRRYGFGSF	NLSSYDVVYS
451	DHCFSVNSDF	CPCADPSVVN	SCAKSKPPSA	ICPAGTKYRH	CDLDTTLYVK
501	NWCRCSCLPD	PISTYSPNTC	PQKKVVVGIG	EHCPGLGINE	EKCGTQLNHS
551	SCFCSPDAFL	GWSFDSCISN	NRCNIFSNFI	FNGINSGTTC	SNDLLYSNTE
601	ISTGVCVNYD	LYGITGQGIF	KEVSAAYYNN	WQNLLYDSNG	NIIGFKDFLT
651	NKTYTILPCY	SGRVSAAFYQ	NSSSPALLYR	NLKCSYVLNN	ISFISQPFYF
701	DSYLGCVLNA	VNLTSYSVSS	CDLRMGSGFC	IDYALPSSGG	SGSGISSPYR
751	FVTFEPFNVS	FVNDSVETVG	GLFEIQIPTN	FTIAGHEEFI	QTSSPKVTID
801	CSAFVCSNYA	ACHDLLSEYG	TFCDNINSIL	NEVNDLLDIT	QLQVANALMQ
851	GVTLSSNLNT	NLHSDVDNID	FKSLLGCLGS	QCGSSSRSLL	EDLLFNKVKL
901	SDVGFVEAYN	NCTGGSEIRD	LLCVQSFNGI	KVLPPILSET	QISGYTTAAT
951	VAAMFPPWSA	AAGVPFSLNV	QYRINGLGVT	MDVLNKNQKL	IANAFNKALL
1001	SIQNGFTATN	SALAKIQSVV	NANAQALNSL	LQQLFNKFGA	ISSSLQEILS
1051	RLDPPEAQVQ	IDRLINGRLT	ALNAYVSQQL	SDITLIKAGA	SRAIEKVNEC
1101	VKSQSPRINF	CGNGNHILSL	VQNAPYGLLF	IHFSYKPTSF	KTVLVSPGLC
1151	LSGDRGIAPK	QGYFIKQNDS	WMFTGSSYYY	PEPISDKNVV	FMNSCSVNFT
1201	KAPFIYLNNS	IPNLSDFEAE	LSLWFKNHTS	IAPNLTFNSH	INATFLDLYY
1251	EMNVIQESIK	SLNS GRLVPR	GSPGSGYIPE	APRDGQAYVR	KDGEWVLLST
1301	FLGHHHHHHG	LNDIFEAQKI	EWHE		

Spike ectodomain – Residues 1 to 1264 (representing residues 14 to 1277) RRKRR to GGSGS substitution of S1/S2 cleavage site – Residues 739 to 743 NL to PP stabilizing mutations – Residues 1054 and 1055 Thrombin cleavage site – Residues 1267 to 1272 T4 foldon trimerization domain – Residues 1276 to 1302 Hexa-histidine tag and AviTag<sup>™</sup> – <u>Residues 1304 to 1324</u>