Spike Glycoprotein S1 Domain from SARS-Related Coronavirus 2, D614G Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells

Catalog No. NR-55418
ACROBiosystems Catalog No. S1N-C5256

For research use only. Not for use in humans.

Contributor and Manufacturer:
ACROBiosystems, Newark, Delaware, USA

Product Description:
A recombinant form of the spike (S) glycoprotein S1 domain from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), D614G variant was produced by transient transfection in human embryonic kidney HEK293 cells and purified by affinity chromatography.[1] NR-55418 lacks the signal sequence, contains 670 residues of the SARS-CoV-2 S glycoprotein (amino acid residues V16 to R685) and features a C-terminal poly-histidine tag. NR-55418 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: QHD43416).[1,2] The predicted protein sequence is shown in Figure 1.[1] NR-55418 has a theoretical molecular weight of 76,800 daltons. The crystal structure for the wild-type S glycoprotein from SARS-CoV-2 has been solved at 2.8 Å resolution (PDB: 6VXX).[3] Representative SDS-PAGE results are shown in Figure 2.[1]

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 100 µg of purified recombinant protein lyophilized in phosphate-buffered saline, pH 7.4 and 10% trehalose.

Packaging/Storage:
NR-55418 was packaged aseptically in glass vials. The product is provided lyophilized and should be placed in a closed, dry environment with desiccants and stored at -20ºC or colder immediately upon arrival. A frost-free freezer should be avoided, since changes in moisture and temperature may affect protein stability.

Functional Activity:
The biological activity of NR-55418 was measured by its binding ability in a functional ELISA (Figure 3), in which immobilized NR-55418 at 2 µg per mL (100 µL per well) can bind human ACE2 protein (Fc tag) (ACROBiosystems AC2-H5257); the linear range is 0.2 to 3 ng per mL.[1] Immobilized NR-55418 at 2 µg per mL (100 µL per well) can also bind Anti-SARS-CoV-2 neutralizing antibody (ACROBiosystems SAD-S35); the linear range is 0.2 to 3 ng per mL (Figure 4).[1] The biological activity of NR-55418 was also measured by its binding ability using biosensor analysis, in which loaded ACROBiosystems AC2-H5257 can bind NR-55418; the affinity constant is 76.4 nM by ForteBio Octet Red96e (Figure 5).[1]

Reconstitution:
NR-55418 should be reconstituted with 250 µL sterile deionized water to a stock solution of 400 µg per mL. Add water at room temperature with occasional gentle mixing. Carrier protein [e.g. 0.1% (w/v) bovine serum albumin] must be included in the reconstitution buffer if the final protein concentration is lower than recommended or NR-55418 is aliquoted to less than 10 µg per vial. Note: Avoid vigorous shaking or vortexing.

Storage of Reconstituted Protein:
Reconstituted NR-55418 should be stored at -70°C or colder immediately and used within 3 months. Avoid repeated freeze-thaw cycles.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Spike Glycoprotein S1 Domain from SARS-Related Coronavirus 2, D614G Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells, NR-55418."

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.

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References:

1. Chen, J., Personal Communication.
2. Wu, F., et al. "A New Coronavirus Associated with Human Respiratory Disease in China." Nature 579 (2020): 265-269. PubMed: 32015508.
3. Walls, A. C., et al. "Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein." Cell 181 (2020): 281-292. PubMed: 32155444.
4. Hulswit, R. J. G., C. A. M. de Haan and B. -J. Bosch. "Coronavirus Spike Protein and Tropism Changes." Adv. Virus Res. 96 (2016): 29-57. PubMed: 27712627.
5. 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." Lancet 397 (2021): 1351-1362. PubMed: 33798499.
6. Klumpp-Thomas, C., et al. "Effect of D614G Spike Variant on Immunoglobulin G, M, or A Spike Seroassay Performance." J. Infect. Dis. 223 (2021): 802-804. PubMed: 33257936.

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Figure 1: Predicted Protein Sequence

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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 TQTNSPRRAR GGGSGGGSHH HHHHHHHH
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S1 domain – **Residues 1 to 670** (represents amino acid residues 16 to 685)
D614G mutation – **Residue 599**
Poly-histidine tag – Residues 679 to 688

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Figure 2: Representative SDS-PAGE

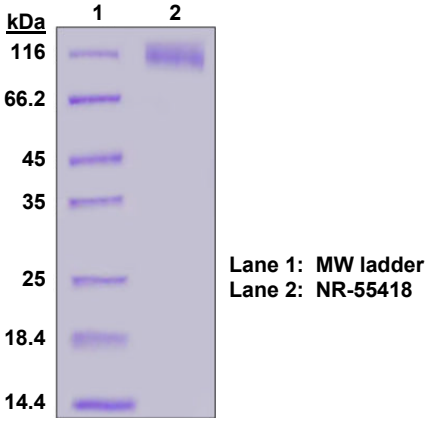


Figure 3: Representative ELISA

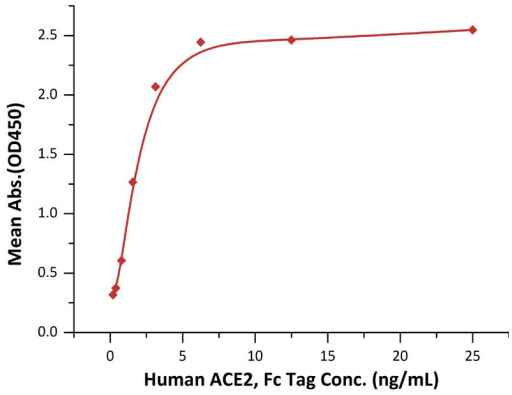


Figure 4: Representative ELISA

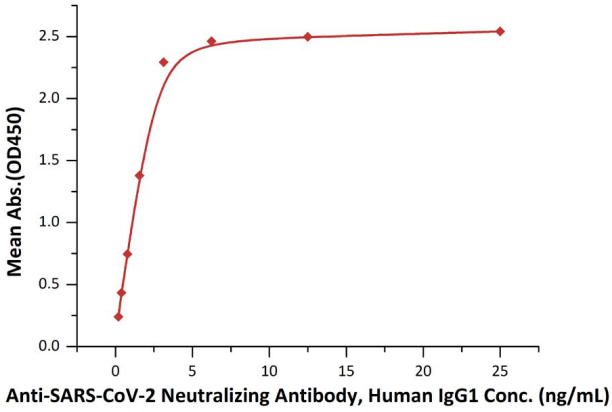
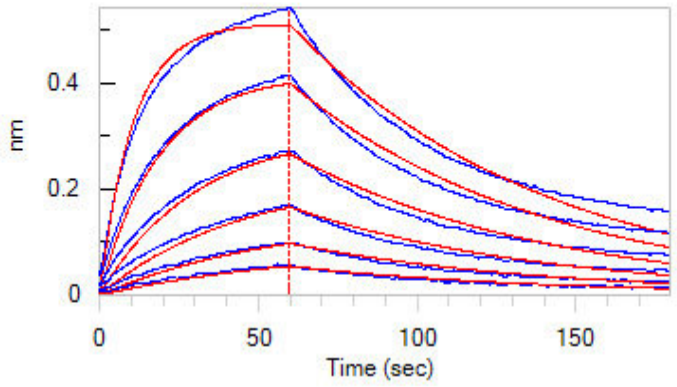


Figure 5: Representative Bioactivity



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