

Spike Glycoprotein Receptor Binding Domain (RBD) from SARS-Related Coronavirus 2, K417N/E484K/N501Y Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells

Catalog No. NR-55414

ACROBiosystems Catalog No. SPD-C52Hp

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Contributor and Manufacturer:

ACROBiosystems, Newark, Delaware, USA

Product Description:

A recombinant form of the spike (S) glycoprotein receptor binding domain (RBD) from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), K417N/E484K/N501Y variant was produced by transient transfection in human embryonic kidney HEK293 cells and purified by affinity chromatography.¹ NR-55414 lacks the signal sequence, contains 219 residues of the SARS-CoV-2 S glycoprotein (amino acid residues R319 to K537) and features a C-terminal poly-histidine tag. NR-55414 is a variant of SARS-CoV-2 which contains the mutations K417N, E484K and N501Y 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-55414 has a theoretical molecular weight of approximately 26,600 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 gel filtration (SEC-MALS) and SDS-PAGE results are shown in Figures 2 and 3, respectively.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 New SARS-CoV-2 mutations in the S glycoprotein are currently under study, and the South African variant (also known as 20C/501Y.V2 or B.1.351 lineage) includes three mutations in the RBD that may have functional significance, K417N, E484K and N501Y.⁵ Structural modeling and mouse studies indicate N501Y increases S glycoprotein binding to ACE2, resulting in increased SARS-CoV-2 virulence.^{6,7} In addition, the E484K mutation has been identified in escape mutants for convalescent antisera.⁸

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-55414 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 -20°C

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-55414 was measured by its binding ability in a functional ELISA (Figure 4), in which immobilized NR-55414 at 1 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-55414 at 1 µg per mL (100 µL per well) can bind anti-SARS-CoV-2 RBD potent neutralizing antibody, human IgG1 (ACROBiosystems SPD-M128); the linear range is 0.1 to 0.8 ng per mL (Figure 5).¹

Reconstitution:

NR-55414 should be reconstituted with 167 µL sterile deionized water to a stock solution of 600 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-55414 is aliquoted to less than 10 µg per vial. Note: Avoid vigorous shaking or vortexing.

Storage of Reconstituted Protein:

Reconstituted NR-55414 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 Receptor Binding Domain (RBD) from SARS-Related Coronavirus 2, K417N/E484K/N501Y Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells, NR-55414.”

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.

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Figure 1: Predicted Protein Sequence

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1  RVQPTESIVR FPNITNLCPF GEVFNATRFA SVYAWNRKRI SNCVADYSVL
51  YNSASFSTFK CYGVSPTKLN DLCFTNVYAD SFVIRGDEVR QIAPGQTGNI
101 ADYNYKLPDD FTGCVIAWNS NNLDSKVGGN YNYLYRLFRK SNLKPFERDI
151 STEIYQAGST PCNGVKGFNC YFPLQSYGFQ PTYGVGYQPY RVVVLSFELL
201 HAPATVCGPK KSTNLVKNKG GSGGGSHHH HHHHHHHH
    
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RBD – Residues 1 to 219 (represents amino acid residues 319 to 537)
 K417N, E484K and N501Y mutations – **Residues 99, 166 and 183**
 Poly-histidine tag – **Residues 228 to 237**

Figure 2: Representative SEC-MALS

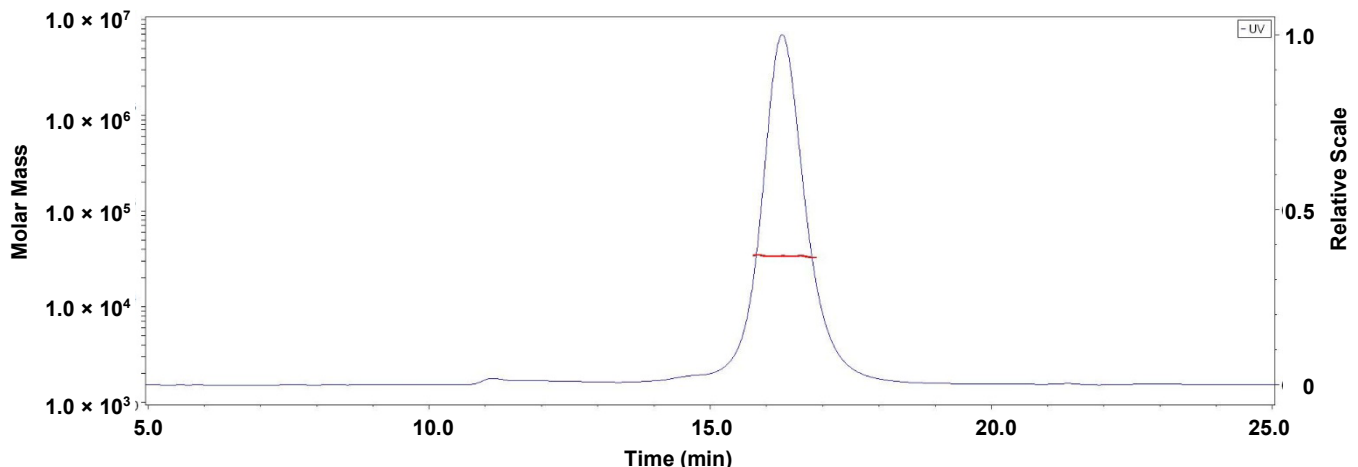


Figure 3: Representative SDS-PAGE

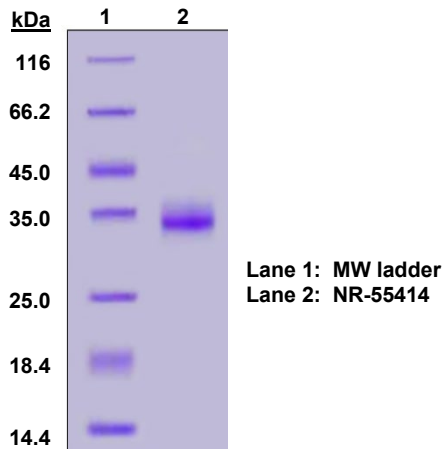


Figure 4: Representative ELISA

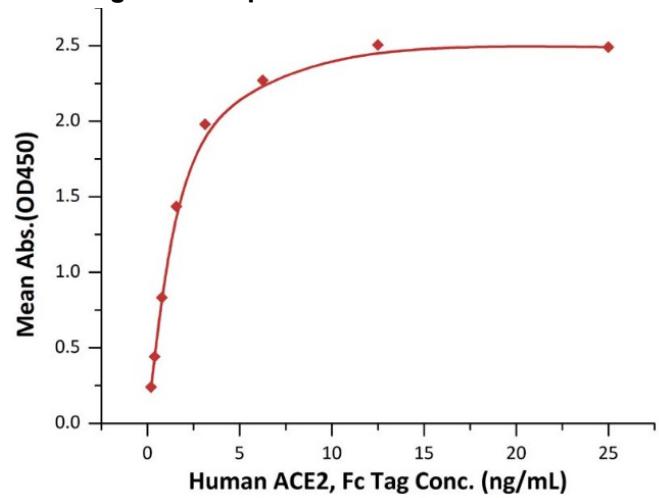


Figure 5: Representative ELISA

