

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

Catalog No. NR-55400

ACROBiosystems Catalog No. SRD-C52H3

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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), E484K variant was produced by transient transfection in human embryonic kidney HEK293 cells and purified by affinity chromatography.¹ NR-55400 lacks the signal sequence, contains 219 residues of the SARS-CoV-2 S glycoprotein RBD (amino acid residues R319 to K537) and features a C-terminal poly-histidine tag. NR-55400 is from a variant of SARS-CoV-2 which contains the E484K 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-55400 has a theoretical molecular weight of 26,500 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 That of SARS-CoV-2 spike RBD with ACE2 has been solved at 2.45 Å resolution (PDB: [6MOJ](#)).⁴

Representative SDS-PAGE, Size Exclusion Chromatography with Multi-Angle Light Scattering (SEC-MALS) analysis and ELISA results are shown in Figures 2 to 5.¹

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.⁵ The E484K mutation was identified in the SARS-CoV-2 variant known as 20C/501Y.V2 or B.1.351 lineage which emerged in South Africa.¹ The E484K mutation in RBD has also been identified globally in many other variants, and increases the affinity of binding to ACE2 and decreases the binding of neutralizing antibodies leading to partial immune escape favoring reinfections.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-55400 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-55400 was measured by its binding ability in a functional ELISA (Figure 4), in which immobilized human ACE2 protein (Fc tag) (ACROBiosystems AC2-H5257) at 1 µg per mL (100 µL per well) can bind NR-55400; the linear range is 0.01 to 0.156 ng per mL.¹ The biological activity of NR-55400 was measured in a functional ELISA in which serial dilutions of anti-SARS-CoV-2, human IgG (ACROBiosystems SAD-S35) were added to NR-55400 and biotinylated human ACE2, His, Avitag™ (ACROBiosystems AC2-H82E6) binding reactions. The half maximal inhibitory concentration (IC₅₀) is 0.82098 µg per mL.

Reconstitution:

NR-55400 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-55400 is aliquoted to less than 10 µg per vial. **Note:** Avoid vigorous shaking or vortexing.

Storage of Reconstituted Protein:

Reconstituted NR-55400 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, E484K Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells, NR-55400."

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. Lan, J., et al. "Structure of the SARS-CoV-2 Spike Receptor-Binding Domain Bound to the ACE2 Receptor." *Nature* 581 (2020): 215-220. PubMed: 32225176.
5. 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.
6. Boehm, E. et al. "Novel SARS-CoV-2 Variants: The Pandemics within the Pandemic." *Clin. Microbiol. Infect.* doi:10.1016/j.cmi.2021.05.022. PubMed: 34015535.

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Figure 1: Predicted Protein Sequence

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1  RVQPTESIVR FPNITNLCPF GEVFNATRFA SVYAWNKRRI SNCVADYSVL
51  YNSASFSTFK CYGVSPSTKLN DLCFTNVYAD SFVIRGDEV R QIAPGQTGKI
101 ADYNYKLDD FTGCVIAWNS NNLD SKVGGN YNYLYRLFRK SNLKPFRDI
151 STEIYQAGST PCNGVKGFNC YFPLQSYGFQ PTNGVGYQPY RVVVL SFELL
201 HAPATVCGPK KSTNLVKNKG GSGGGSHHH HHHHHHH

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RBD – Residues 1 to 219 (represents amino acid residues 319 to 537)

E484K mutation – **Residue 166**

Poly-histidine tag – Residues 228 to 237

Figure 2: Representative SDS-PAGE

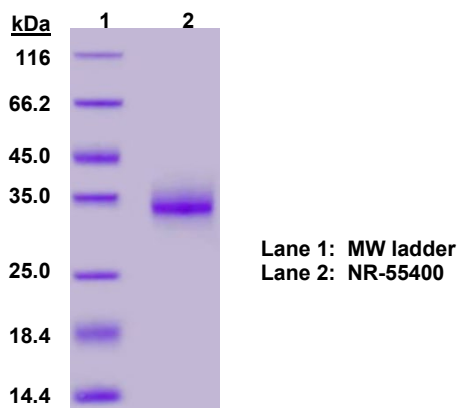


Figure 3: Representative SEC-MALS

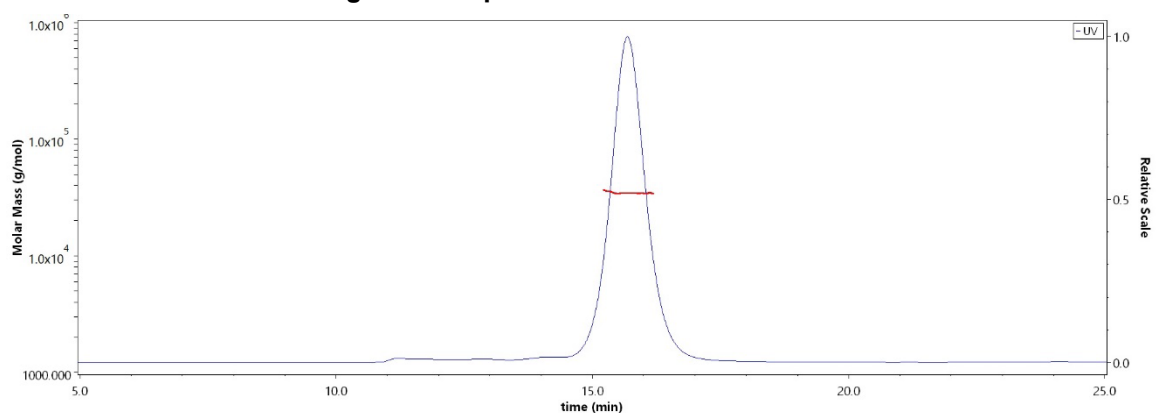


Figure 4: Representative ELISA

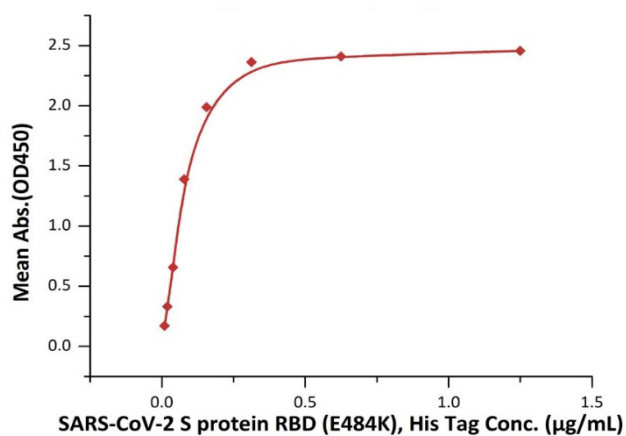


Figure 5: Representative ELISA

