

**Spike Glycoprotein Receptor Binding Domain (RBD) from SARS-Related Coronavirus 2, N440K Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells**

**Catalog No. NR-55405**  
**ACROBiosystems Catalog No. SRD-C52H2**

**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 receptor binding domain (RBD) from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), N440K variant was produced by transient transfection in human embryonic kidney HEK293 cells and purified by affinity chromatography.<sup>1</sup> NR-55405 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-55405 is a variant of SARS-CoV-2 which contains the N440K mutation in the S glycoprotein as compared to the SARS-CoV-2 reference sequence (GenPept: [QHD43416](#)).<sup>1,2</sup> The predicted protein sequence is shown in Figure 1.<sup>1</sup> NR-55405 has a theoretical molecular weight of 26,500 daltons. Representative SDS-PAGE results are shown in Figure 2.<sup>1</sup>

Representative SDS-PAGE, Size Exclusion Chromatography with Multi-Angle Light Scattering (SEC-MALS) analysis and ELISA results are shown in Figures 2 to 5.<sup>1</sup>

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>3</sup> The N440K mutation results in higher affinity of S to the human ACE2 receptor.<sup>4</sup>

**Material Provided:**

Each vial contains approximately 100 micrograms of purified recombinant protein lyophilized in phosphate-buffered saline, pH 7.4 and 10% trehalose.

**Packaging/Storage:**

NR-55405 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-55405 was measured by its binding ability in a functional ELISA (Figure 4), in which

immobilized NR-55405 at 1 microgram per milliliter (100 microliters per well) can bind human ACE2 protein (Fc tag) (ACROBiosystems AC2-H5257); the linear range is 10 to 78 nanograms per milliliter.<sup>1</sup> The biological activity of NR-55405 was measured by its binding ability in a functional ELISA (Figure 5), in which immobilized Anti-SARS-CoV-2 RBD Neutralizing Antibody, Human IgG1 (ACROBiosystems SAD-S35) at 1 microgram per milliliter (100 microliters per well) can bind NR-55405 with a linear range of 5 to 39 nanograms per milliliter.<sup>1</sup>

**Reconstitution:**

NR-55405 should be reconstituted with 167 microliters sterile deionized water to a stock solution of 600 micrograms per milliliter. 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-55405 is aliquoted to less than 10 micrograms per vial. Note: Avoid vigorous shaking or vortexing.

**Storage of Reconstituted Protein:**

Reconstituted NR-55405 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, N440K Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells, NR-55405.”

**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](http://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. 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.
4. Gan, H. H., et al. "Structural Modeling of the SARS-CoV-2 Spike/Human ACE2 Complex Interface can Identify High-Affinity Variants Associated with Increased Transmissibility." *J. Mol. Biol.* 433 (15): 167051. PubMed: 33992693.

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**Figure 1: Predicted Protein Sequence**

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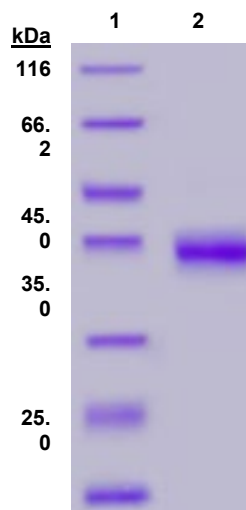
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51  YNSASFSTFK CYGVSPTKLN DLCFTNVYAD SFVIRGDEV R QIAPGQTGKI
101 ADYNYKLPDD FTGCVIAWNS NKLDSKVGGN YNYLYRLFRK SNLKPFERDI
151 STEIYQAGST PCNGVEGFNC YFPLQSYGFQ PTNGVGYQPY RVVVLSFELL
201 HAPATVCGPK KSTNLVKKNG GSGGGGSHHH HHHHHHHH
    
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RBD domain – **Residues 1 to 219** (represents amino acid residues 319 to 537)

N440K mutation – **Residue 122**

Poly-histidine tag – **Residues 228 to 237**

**Figure 2: Representative SDS-PAGE**



Lane 1: MW ladder  
Lane 2: NR-55405

Figure 3: Representative SEC-MALS

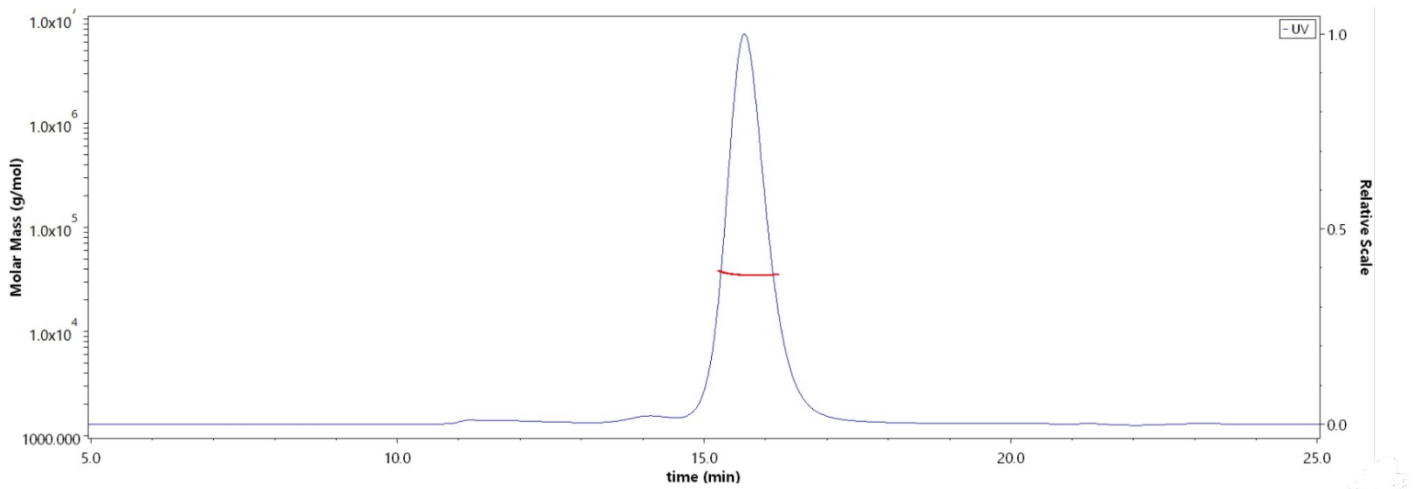


Figure 4: Representative ELISA

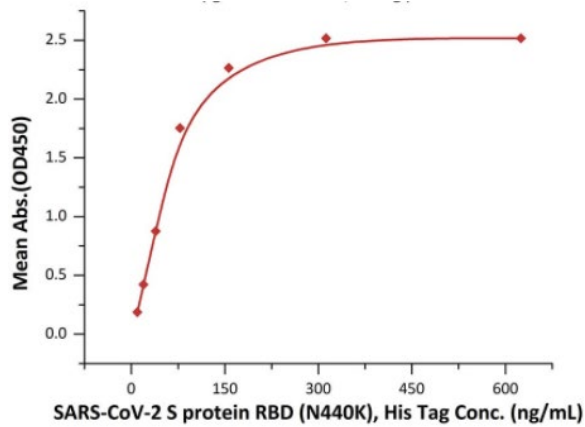


Figure 5: Representative ELISA

