

**Spike Glycoprotein Receptor Binding Domain (RBD) from SARS-Related Coronavirus 2, A475V Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells**

**Catalog No. NR-55402**

**ACROBiosystems Catalog No. SPD-C52Hd**

**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), A475V variant was produced by transient transfection in human embryonic kidney HEK293 cells and purified by affinity chromatography.<sup>1</sup> NR-55402 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-55402 is from a variant of SARS-CoV-2 which contains the A475V 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-55402 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](#)).<sup>3</sup> The crystal structure of SARS-CoV-2 spike RBD with ACE2 has been solved at 2.45 Å resolution (PDB: [6M0J](#)).<sup>4</sup>

Representative SDS-PAGE, ELISA, Surface Plasmon Resonance (SPR) and Bio-Layer Interferometry (BLI) analysis results are shown in Figures 2 to 9.<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>5</sup> The A475V mutation is widespread and was reported to reduce spike protein sensitivity to neutralizing antibodies.<sup>6</sup>

**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-55402 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-55402 was measured by its binding ability in a functional ELISA (Figure 3), in which immobilized NR-55402 at 1 µg per mL (100 µL per well) can bind human ACE2 protein (Fc tag) (ACROBiosystems AC2-H5257); the linear range is 0.1 to 3 ng per mL.<sup>1</sup> The biological activity of NR-55402 was measured in a functional ELISA (Figure 4), in which serial dilutions of Anti-SARS-CoV-2, human IgG (ACROBiosystems SAD-S35) were added to NR-55402 and biotinylated human ACE2, His, Avitag™ (ACROBiosystems AC2-H82E6) binding reactions. The half maximal inhibitory concentration (IC<sub>50</sub>) is 19.5 µg per mL.<sup>1</sup>

The sensitivity of NR-55402 to neutralizing antibodies was measured by a functional ELISA (Figure 5), in which serial dilutions of Anti-SARS-CoV-2 RBD Neutralizing Antibody, Human IgG1 (ACROBiosystems SAD-S35) were added into NR-55402 and SARS-CoV-2 (COVID-19) S protein RBD, His Tag (ACROBiosystems SPD-C52H3), Biotinylated Human ACE2 Protein, His, Avitag™ (ACROBiosystems AC2-H82E6) binding reactions. The IC<sub>50</sub> of NR-55402 and ACROBiosystems SPD-C52H3 are 4.67291 µg per mL and 1.44469 µg per mL respectively.

The sensitivity of NR-55402 to neutralizing antibodies was measured by a functional ELISA (Figure 6), in which serial dilutions of Anti-SARS-CoV-2 RBD Potent Neutralizing Antibody, Chimeric mAb, Human IgG1 (ACROBiosystems SPD-M128) were added into NR-55402 and SARS-CoV-2 (COVID-19) S protein RBD, His Tag (ACROBiosystems SPD-C52H3), Biotinylated Human ACE2 Protein, His, Avitag™ (ACROBiosystems AC2-H82E6) binding reactions. The IC<sub>50</sub> of NR-55402 and ACROBiosystems SPD-C52H3 are 0.26478 µg per mL and 0.78328 µg per mL respectively.

The biological activity of NR-55402 was also measured by its binding ability using biosensor analysis, in which human ACE2 protein (Fc tag) (ACROBiosystems AC2-H5257) or Anti-SARS-CoV-2 RBD Neutralizing Antibody, Human IgG1 (Cat. No. SAD-S35) can bind NR-55402; the affinity constant is 25.8 nM and 87.7 nM, respectively by Biacore T200 (Figures 7 and 8). NR-55402 can bind ACROBiosystems AC2-H5257 with an affinity constant of 11 nM by ForteBio Octet Red96e (Figure 9).<sup>1</sup>

**Reconstitution:**

NR-55402 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-55402 is aliquoted to less than 10 µg per vial. Note: Avoid vigorous shaking or vortexing.

**Storage of Reconstituted Protein:**

Reconstituted NR-55402 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, A475V Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells, NR-55402.”

**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. 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. Li, Q., et al. “The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity.” Cell 182 (2020): 1284-1294.e9. PubMed: 32730807.

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**Figure 1: Predicted Protein Sequence**

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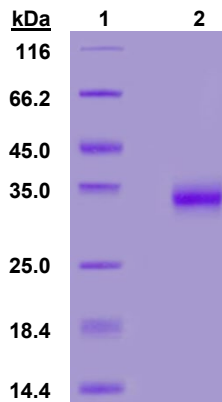
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51  YNSASFSTFK CYGVSPTKLN DLCFTNVYAD SFVIRGDEVK QIAPGQTGKI
101 ADYNYKLDD FTGCVIAWNS NNLDKVGGN YNYLYRLFRK SNLKPFRDI
151 STEIYQVGS PCNGVEGFNC YFPLQSYGFQ PTNGVGYQPY RVVVLSFELL
201 HAPATVCGPK KSTNLVKNKG GSGGGSHHH HHHHHHH
    
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RBD – Residues 1 to 219 (represents amino acid residues 319 to 537)

A475V mutation – **Residue 157**

Poly-histidine tag – Residues 228 to 237

Figure 2: Representative SDS-PAGE



Lane 1: MW ladder  
Lane 2: NR-55402

Figure 3: Representative ELISA

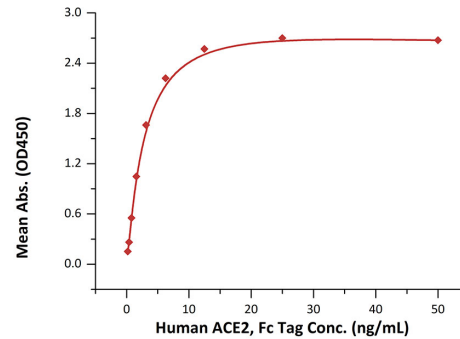


Figure 4: Representative ELISA

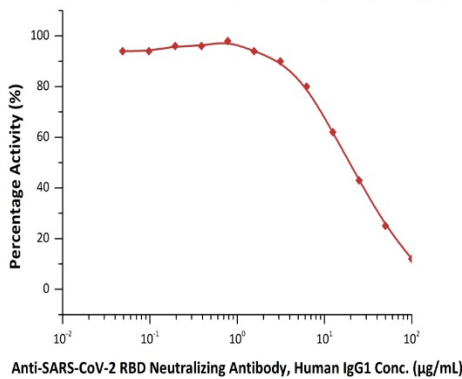


Figure 5: Representative ELISA

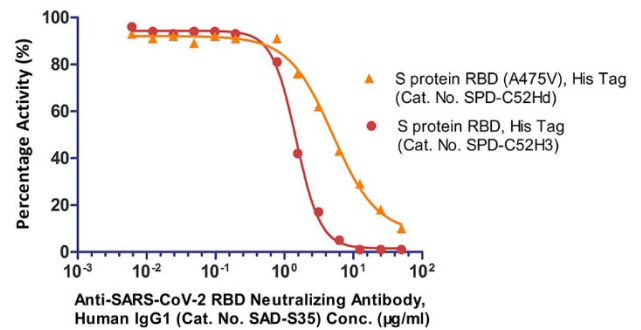


Figure 6: Representative ELISA

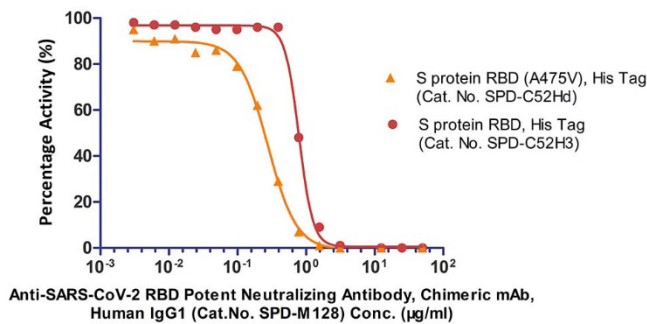


Figure 7: Representative SPR Analysis

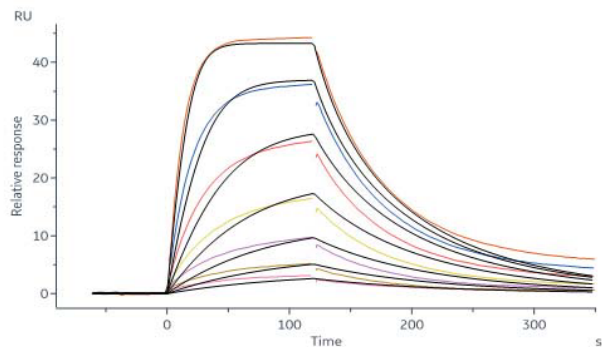


Figure 8: Representative SPR Analysis

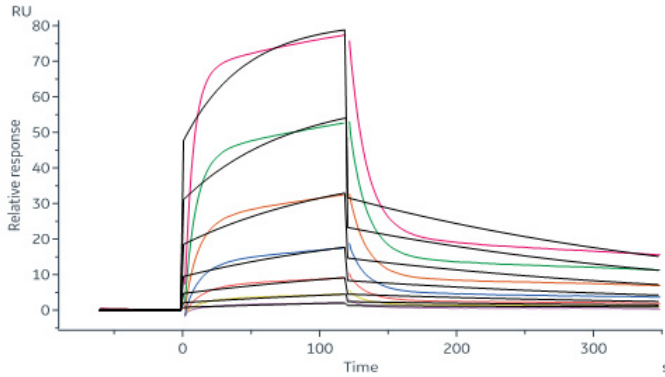


Figure 9: Representative BLI Analysis

