

Spike Glycoprotein Receptor Binding Domain (RBD) from SARS-Related Coronavirus 2, G476S Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells

Catalog No. NR-55401

ACROBiosystems Catalog No. SPD-C52H4

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), G476S variant was produced by transient transfection in human embryonic kidney HEK293 cells and purified by affinity chromatography.¹ NR-55401 lacks the signal sequence, contains 223 residues of the SARS-CoV-2 S glycoprotein RBD (amino acid residues R319 to F541) and features a C-terminal poly-histidine tag. NR-55401 is from a variant of SARS-CoV-2 which contains the G476S 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-55401 has a theoretical molecular weight of 27,000 daltons. The crystal structure for the wild-type S glycoprotein from SARS-CoV-2 has been solved at 2.8 Å resolution (PDB: [6VXX](#)) and that of SARS-CoV-2 spike RBD with ACE2 has been solved at 2.45 Å resolution (PDB: [6MOJ](#)).^{3,4}

Representative SDS-PAGE, ELISA, Surface Plasmon Resonance (SPR) and Bio-Layer Interferometry (BLI) analysis results are shown in Figures 2 to 6.¹

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 G476S mutation is widespread and was identified in multiple viral strains. The G476S mutation was reported to decrease the affinity of binding to ACE2.⁶

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-55401 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-55401 was measured by its binding ability in a functional ELISA (Figure 3), in which immobilized human ACE2 protein (Fc tag) (ACROBiosystems AC2-H5257) at 1 µg per mL (100 µL per well) can bind NR-55401; the linear range is 2 to 16 ng per mL.¹ The biological activity of NR-55401 was measured in a functional ELISA (Figure 4), in which anti-SARS-CoV-2, human IgG (AcroBiosystems SAD-S35) can bind NR-55401. The linear range is 2 to 8 ng per mL.¹ The biological activity of NR-55401 was also measured by its binding ability using biosensor analysis, in which human ACE2 protein (Fc tag) (ACROBiosystems AC2-H5257) can bind NR-55401; the affinity constant is 5.03 nM by Biacore T200 (Figure 5) and 5.49 nM by ForteBio Octet Red96e (Figure 6).¹

Reconstitution:

NR-55401 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-55401 is aliquoted to less than 10 µg per vial. Note: Avoid vigorous shaking or vortexing.

Storage of Reconstituted Protein:

Reconstituted NR-55401 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, G476S Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells, NR-55401.”

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/bmb15/index.htm.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for

informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

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. Chakraborty, S. "Evolutionary and Structural Analysis Elucidates Mutations on SARS-CoV-2 Spike Protein with Altered Human ACE2 Binding Affinity." *Biochem. Biophys. Res. Commun.* 534 (2021): 374-380. PubMed: 33602511.

ATCC® is a trademark of the American Type Culture Collection.



Figure 1: Predicted Protein Sequence

```

1  RVQPTESIVR FPNITNLCPF GEVFNATRFA SVYAWNRRKRI SNCVADYSVL
51  YNSASFSTFK CYGVSPTKLN DLCFTNVYAD SFVIRGDEV R QIAPGQTGKI
101 ADYNYKLPDD FTGCVIAWNS NNLDSKVGGN YNYLYRLFRK SNLKPFERDI
151 STEIYQASST PCNGVEGFNC YFPLQSYGFQ PTNGVGYQPY RVVVLSFELL
201 HAPATVCGPK KSTNLVKKNC VNFGGGSGGG SHHHHHHHHH H
    
```

RBD – Residues 1 to 223 (represents amino acid residues 319 to 541)
 G476S mutation – **Residue 158**
 Poly-histidine tag – Residues 232 to 241

Figure 2: Representative SDS-PAGE

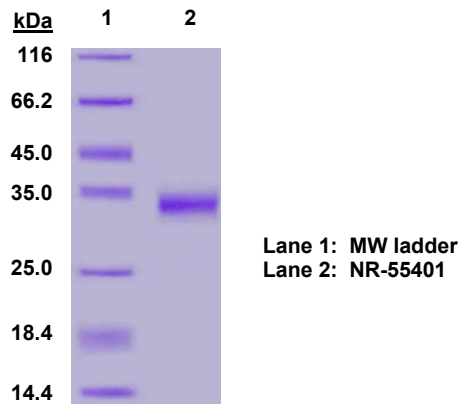


Figure 3: Representative ELISA

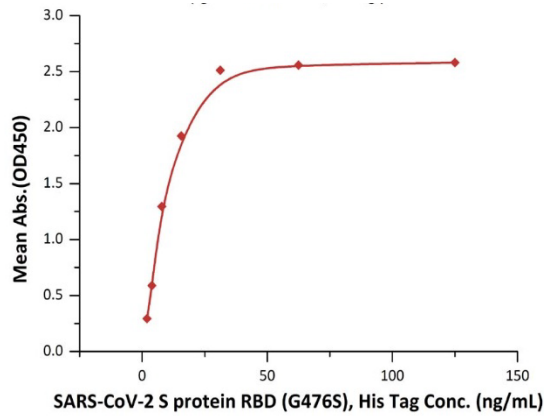


Figure 4: Representative ELISA

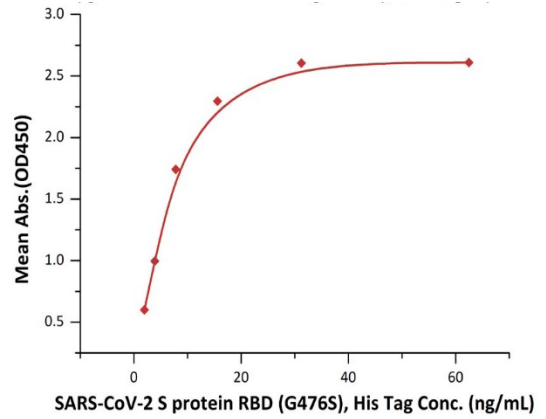


Figure 5: Representative SPR Analysis

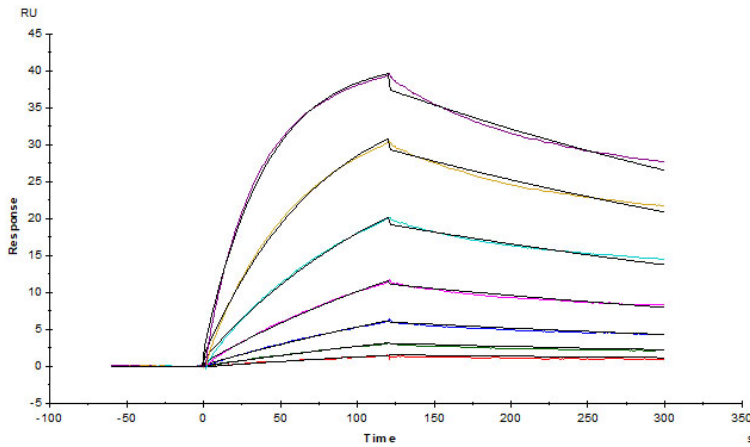


Figure 6: Representative BLI Analysis

