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

Catalog No. NR-55413
ACROBiosystems Catalog No. SPD-C52Hn

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), N501Y variant was produced by transient transfection in human embryonic kidney HEK293 cells and purified by affinity chromatography.[1] NR-55413 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-55413 is a variant of SARS-CoV-2 which contains the N501Y 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-55413 has a theoretical molecular weight of 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] Many SARS-CoV-2 variants include multiple mutations that were first identified in the United Kingdom, and the most studied is N501Y. Structural modeling and mouse studies indicate N501Y increases S glycoprotein binding to ACE2, resulting in increased SARS-CoV-2 virulence.[5,6]

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-55413 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 -20ºC 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-55413 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-55413; the linear range is 2 to 39 ng per mL.[1] Immobilized NR-55413 at 1 µg per mL (100 µL per well) can bind anti-SARS-CoV-2 RBD potent neutralizing antibody, human IgG1 (ACROBiosystems SPD-M180); the linear range is 0.1 to 3 ng per mL (Figure 5).[1] Serial dilutions of SPD-M180 were added to NR-55413 and biotinylated human ACE2, His, Avitag™ (ACROBiosystems AC2-H82E6) binding reactions. The half maximal inhibitory concentration (IC_{50}) is 1.13169 µg per mL (Figure 6).

Reconstitution:
NR-55413 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-55413 is aliquoted to less than 10 µg per vial. Note: Avoid vigorous shaking or vortexing.

Storage of Reconstituted Protein:
Reconstituted NR-55413 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, N501Y Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells, NR-55413."

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.

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

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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. 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.
5. Gu, H., et al. "Adaptation of SARS-CoV-2 in BALB/c Mice for Testing Vaccine Efficacy." Science 369 (2020): 1603-1607. PubMed: 32732280.
6. Leung, K., et al. "Early Transmissibility Assessment of the N501Y Mutant Strains of SARS-CoV-2 in the United Kingdom, October to November 2020." Euro. Surveill. 26 (2021): pii 2002106. PubMed: 33413740.

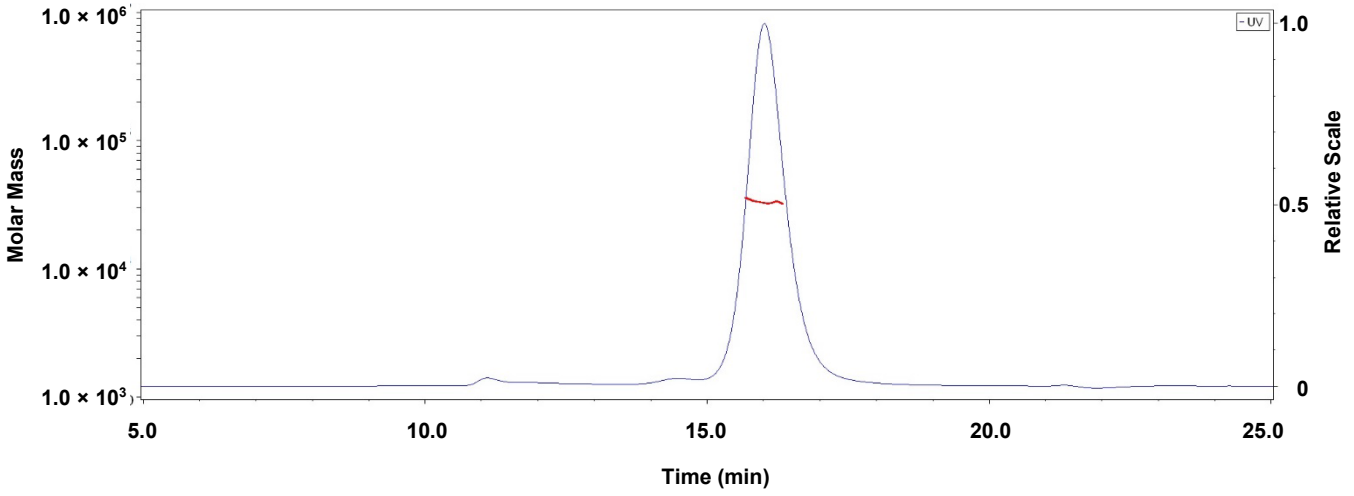
Support Provided by NIAID

Figure 1: Predicted Protein Sequence

1 **RVQPTESIVR FPNITNLCPF GEVFNATRFA SVYAWNRKRI SNCVADYSVL**
51 **YNSASFSTFK CYGVSPTKLN DLCFTNVYAD SFVIRGDEVR QIAPGQTGKI**
101 **ADYNYKLPDD FTGCVIAWNS NNLDSKVGGN YNYLYRLFRK SNLKPFERDI**
151 **STEIYQAGST PCNGVEGFNC YFPLQSYGFQ PTYGVGYQPY RVVVLSFELL**
201 **HAPATVCGPK KSTNLVKNK**G GGSGGGSHHH HHHHHH

RBD – **Residues 1 to 219** (represents amino acid residues 319 to 537)
N501Y mutation – **Residue 183**
Poly-histidine tag – Residues 228 to 237

Figure 2: Representative SEC-MALS



BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Figure 3: Representative SDS-PAGE

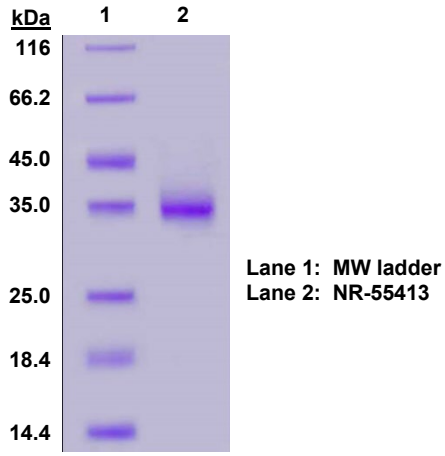


Figure 4: Representative ELISA

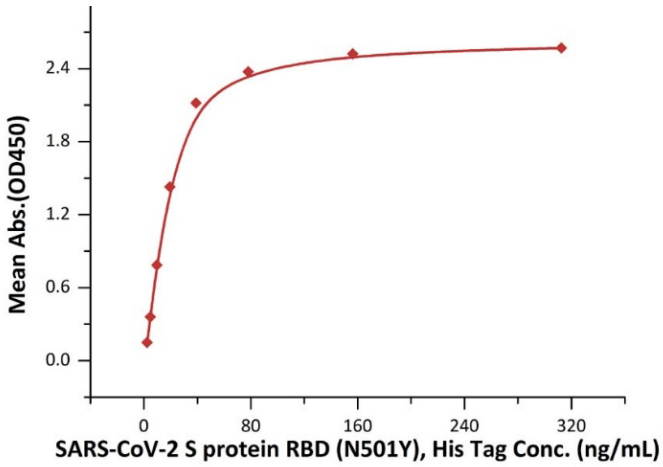


Figure 5: Representative ELISA

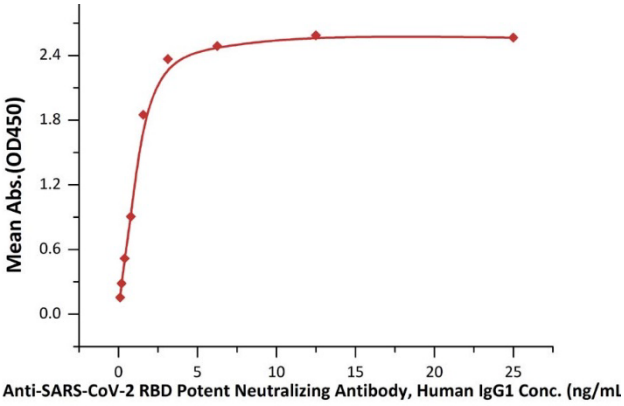
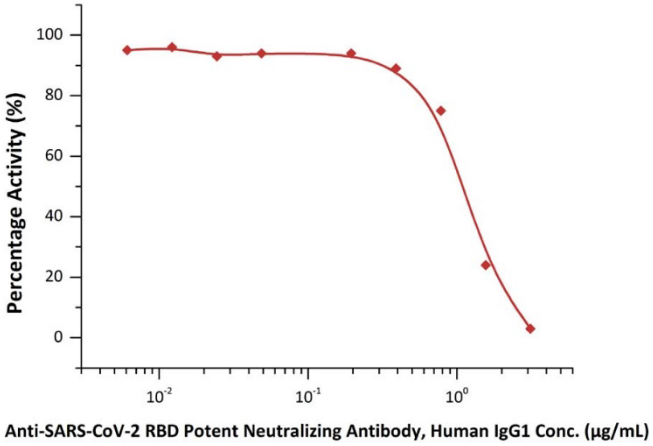


Figure 6: Representative ELISA



BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898