Product Information Sheet for NR-55403

Spike Glycoprotein Receptor Binding Domain (RBD) from SARS-Related Coronavirus 2, L452R Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells

Catalog No. NR-55403
ACROBiosystems Catalog No. SPD-C52He

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), L452R variant was produced by transient transfection in human embryonic kidney HEK293 cells and purified by affinity chromatography.[1] NR-55403 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-55403 is from a variant of SARS-CoV-2 which contains the L452R 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-55403 has a theoretical molecular weight of 26,600 daltons.

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.[3] The L452R mutation has been shown to decrease sensitivity to neutralizing antibodies, increase viral infectivity and enhance viral replication capacity.[4,5]

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-55403 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-55403 was measured by its binding ability in a functional ELISA (Figure 3), in which immobilized NR-55403 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 2 ng per mL.[1]

The biological activity of NR-55403 was also measured by its binding ability using biosensor analysis. 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-55403 with the affinity constants 20.1 nM and 17.4 nM, respectively, using ForteBio Octet Red96e (Figures 4 and 5). NR-55403 can bind ACROBiosystems SAD-S35 with an affinity constant of 19.6 nM by Biacore 8K (Figure 6).[1]

Reconstitution:
NR-55403 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-55403 is aliquoted to less than 10 µg per vial. Note: Avoid vigorous shaking or vortexing.

Storage of Reconstituted Protein:
Reconstituted NR-55403 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, L452R Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells, NR-55403."

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

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

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. 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. Motozono, C., et al. "SARS-CoV-2 Spike L452R Variant Evades Cellular Immunity and Increases Infectivity." Cell Host Microbe 29 (2021): 1124-1136.e11. PubMed: 34171266.
5. 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.

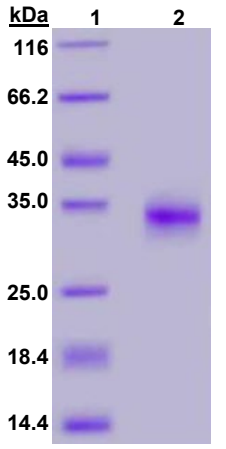
Support Provided by NIAID

Figure 1: Predicted Protein Sequence

```
1   RVQPTESIVR FPNITNLCPF GEVFNATRFA SVYAWNRKRI SNCVADYSVL
51  YNSASFSTFK CYGVSPTKLN DLCFTNVYAD SFVIRGDEVR QIAPGQTGKI
101 ADYNYKLPDD FTGCVIAWNS NNLDSKVGGN YNYRYRLFRK SNLKPFERDI
151 STEIYQAGST PCNGVEGFNC YFPLQSYGFQ PTNGVGYQPY RVVVLSFELL
201 HAPATVCGPK KSTNLVKNKG GGSGGGSHHH HHHHHH
```

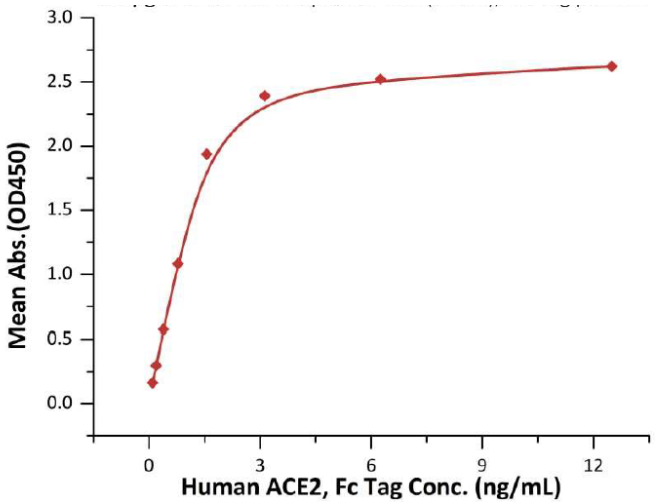
RBD domain – **Residues 1 to 219** (represents amino acid residues 319 to 537)
L452R mutation – **Residue 134**
Poly-histidine tag – Residues 228 to 237

Figure 2: Representative SDS-PAGE



Lane 1: MW ladder
Lane 2: NR-55403

Figure 3: Representative ELISA



BEI Resources
www.beiresources.org

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

Figure 4: Representative Bioactivity-BLI

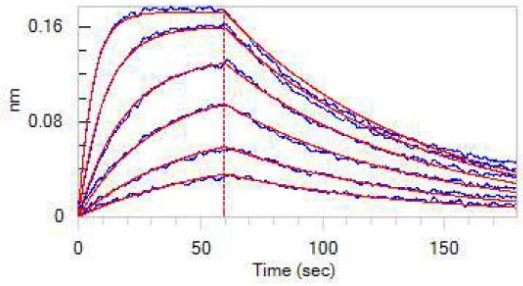


Figure 5: Representative Bioactivity-BLI

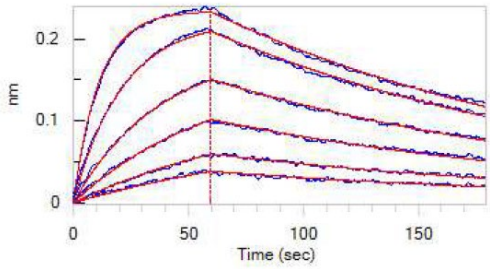
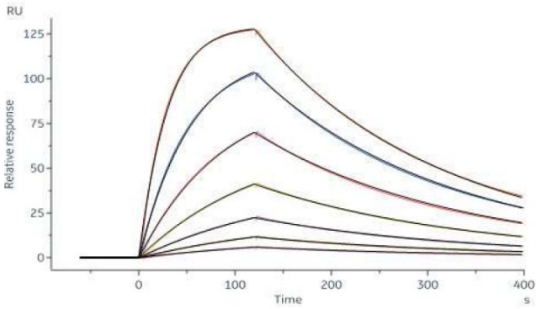


Figure 6: Representative Bioactivity-SPR



BEI Resources
www.beiresources.org

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