

Spike Glycoprotein Receptor Binding Domain (RBD) from SARS-Related Coronavirus 2, V483A Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells

Catalog No. NR-55409

ACROBiosystems Catalog No. SPD-C52H5

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), V483A variant was produced by transient transfection in human embryonic kidney HEK293 cells and purified by affinity chromatography.¹ NR-55409 lacks the signal sequence, contains 223 residues of the SARS-CoV-2 S glycoprotein (amino acid residues R319 to F541) and features a C-terminal poly-histidine tag. NR-55409 is a variant of SARS-CoV-2 which contains the V483A 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-55409 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](#)).³ Representative SDS-PAGE results are shown in Figure 2.¹

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 The V483A mutation removes an S glycoprotein glycosylation site, and SARS-CoV-2 variants with this mutation show resistance to some neutralizing antibodies.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-55409 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-55409 was measured by its binding ability in a functional ELISA (Figure 3), in which immobilized human ACE2 protein (Fc tag) (ACROBiosystems

AC2-H5257) at 5 µg per mL (100 µL per well) can bind NR-55409; the linear range is 2 to 50 ng per mL.¹ Immobilized Anti-SARS-CoV-2 neutralizing antibody (ACROBiosystems SAD-S35) at 1 µg per mL (100 µL per well) can bind NR-55409; the linear range is 2 to 16 ng per mL (Figure 4).¹ The biological activity of NR-55409 was also measured by its binding ability using biosensor analysis, in which loaded ACROBiosystems AC2-H5257 can bind NR-55409; the affinity constant is 4.13 nM by Biocore T200 (Figure 5) and 6.15 nM by ForteBio Octet Red96e (Figure 6). In addition, loaded ACROBiosystems SAD-S35 can bind NR-55409; affinity constant is 4.57 nM by ForteBio Octet Red96e (Figure 7).¹

Reconstitution:

NR-55409 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-55409 is aliquoted to less than 10 µg per vial. **Note:** Avoid vigorous shaking or vortexing.

Storage of Reconstituted Protein:

Reconstituted NR-55409 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, V483A Variant with C-Terminal Histidine Tag, Recombinant from HEK293 Cells, NR-55409."

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.

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

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Figure 1: Predicted Protein Sequence

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1  RVQPTESIVR FPNITNLCPE GEVFNATREA SVYAWNRRKI SNCVADYSVL
51  YNSASFSTFK CYGVSPSTKLN DLCTNTVYAD SFVIRGDEVK QIAPGQTGKI
101 ADYNYKLDD FTGCVIAWNS NNLDKVGGN YNYLYRLFRK SNLKPFFERDI
151 STEIYQAGST PCNGAEGFNC YFPLQSYGFQ PTNGVGYQPY RVVVLSEFLL
201 HAPATVCGPK KSTNLVKNKC VNFGGGSGGG SHHHHHHHHH H

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RBD – Residues 1 to 223 (represents amino acid residues 319 to 541)

V483A mutation – **Residue 165**

Poly-histidine tag – Residues 232 to 241

Figure 2: Representative SDS-PAGE

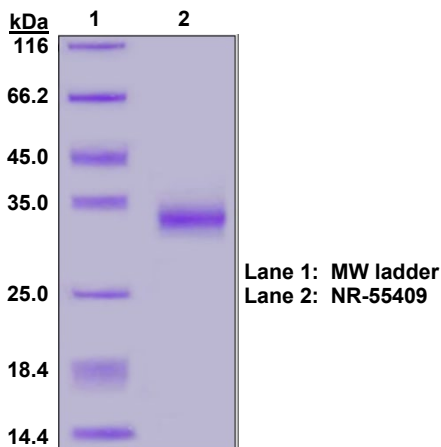


Figure 3: Representative ELISA

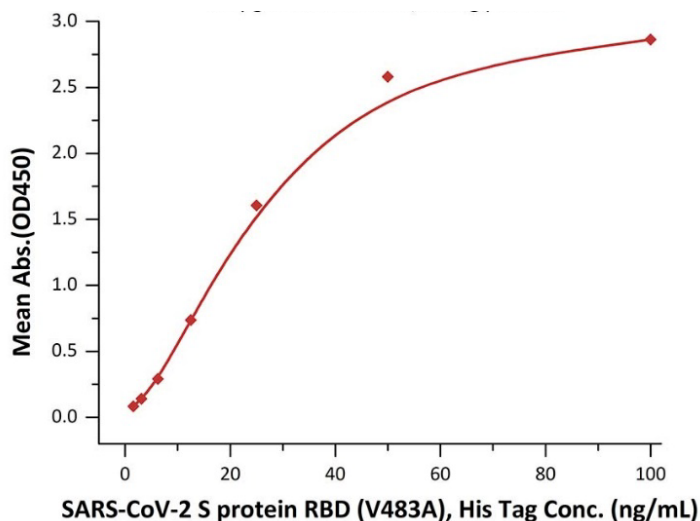


Figure 4: Representative ELISA

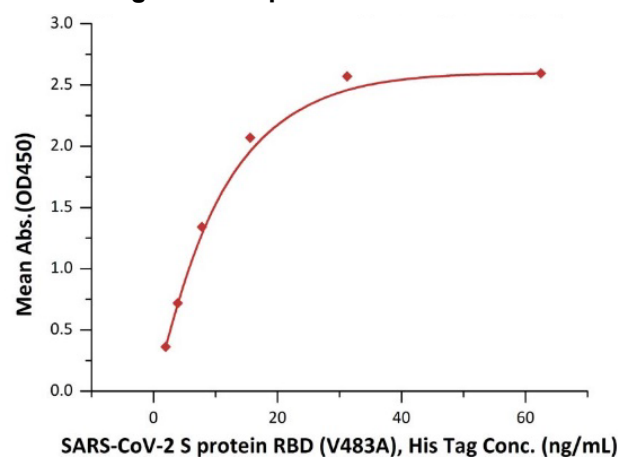


Figure 5: Representative Biosensor

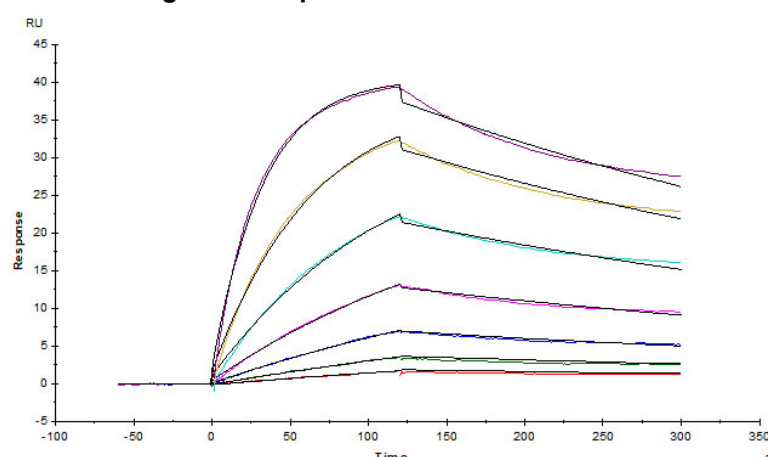


Figure 6: Representative Bioactivity

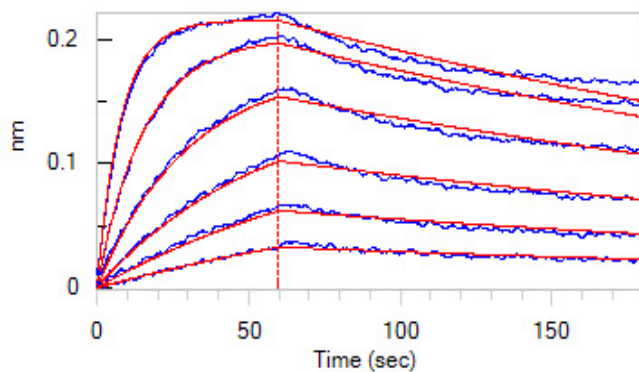


Figure 7: Representative Bioactivity

