

DESCRIPTION

Source Human embryonic kidney cell, HEK293-derived sars-cov-2 Spike RBD protein
Arg319-Phe541 (Asn439Lys), with a C-terminal 6-His tag
Accession # YP_009724390.1

N-terminal Sequence Analysis Arg319

Predicted Molecular Mass 26 kDa

SPECIFICATIONS

SDS-PAGE 33-38 kDa, under reducing conditions

Activity Measured by its binding ability in a functional ELISA with Recombinant Human ACE-2 His-tag (Catalog # 933-ZN).

Endotoxin Level <0.10 EU per 1 µg of the protein by the LAL method.

Purity >95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution Reconstitute at 500 µg/mL in PBS.

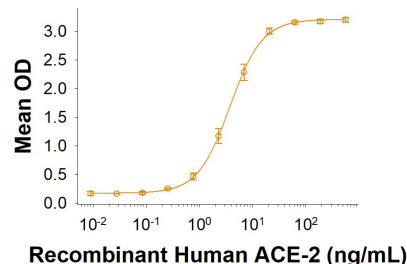
Shipping The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 12 months from date of receipt, -20 to -70 °C as supplied.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 3 months, -20 to -70 °C under sterile conditions after reconstitution.

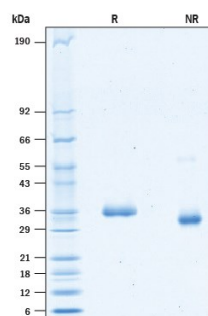
DATA

Bioactivity



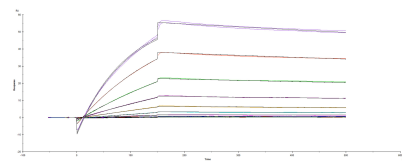
Recombinant SARS-CoV-2 N439K Spike RBD His-tag Protein Bioactivity. Recombinant SARS-CoV-2 N439K His-tag (Catalog # 10714-CV) binds Recombinant Human ACE-2 His-tag (Catalog # 933-ZN) in a functional ELISA.

SDS-PAGE



Recombinant SARS-CoV-2 N439K Spike RBD His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant SARS-CoV-2 N439K Spike RBD His-tag (Catalog # 10714-CV) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 33-38 kDa.

Surface Plasmon Resonance (SPR)



Binding of ACE-2 to SARS-CoV-2 Spike RBD N437K by surface plasmon resonance (SPR). Recombinant SARS-CoV-2 Spike RBD N439K His-tag was immobilized on a Biacore Sensor Chip CM5, and binding to recombinant human ACE-2 (Catalog # 933-ZN) was measured at a concentration range between 0.18 nM and 94.3 nM. The double-referenced sensorgram was fit to a 1:1 binding model to determine the binding kinetics and affinity, with an affinity constant of $K_D=2.805$ nM.

BACKGROUND

SARS-CoV-2, which causes the global pandemic coronavirus disease 2019 (Covid-19), belongs to a family of viruses known as coronaviruses that also include MERS-CoV and SARS-CoV-1. Coronaviruses are commonly comprised of four structural proteins: Spike protein (S), Envelope protein (E), Membrane protein (M) and Nucleocapsid protein (N) (1). The SARS-CoV-2 S protein is a glycoprotein that mediates membrane fusion and viral entry. The S protein is homotrimeric, with each ~180-kDa monomer consisting of two subunits, S1 and S2 (2). In SARS-CoV-2, as with most coronaviruses, proteolytic cleavage of the S protein into S1 and S2 subunits is required for activation. The S1 subunit is focused on attachment of the protein to the host receptor while the S2 subunit is involved with cell fusion (3-5). A receptor binding domain (RBD) in the C-terminus of the S1 subunit has been identified and the RBD of SARS-CoV-2 shares 73% amino acid (aa) identity with the RBD of the SARS-CoV-1, but only 22% aa identity with the RBD of MERS-CoV (6,7). The low aa sequence homology is consistent with the finding that SARS and MERS-CoV bind different cellular receptors (8). The RBD of SARS-CoV-2 binds a metalloproteinase, angiotensin-converting enzyme 2 (ACE-2), similar to SARS-CoV-1, but with much higher affinity and faster binding kinetics (9). Before binding to the ACE-2 receptor, structural analysis of the S1 trimer shows that only one of the three RBD domains is in the "up" conformation. This is an unstable and transient state that passes between trimeric subunits but is nevertheless an exposed state to be targeted for neutralizing antibody therapy (10). Polyclonal antibodies to the RBD of the SARS-CoV-2 protein have been shown to inhibit interaction with the ACE-2 receptor, confirming RBD as an attractive target for vaccinations or antiviral therapy (11). There is also promising work showing that the RBD may be used to detect presence of neutralizing antibodies present in a patient's bloodstream, consistent with developed immunity after exposure to the SARS-CoV-2 (12). As of early 2021, a N439K mutation was observed in 34 countries and was the second most commonly observed RBD mutation worldwide, and the sixth most common S mutation. The N439K mutation has been identified with modestly enhanced RBD affinity for hACE-2 and slightly higher viral loads in vivo compared to viruses with the wild-type N439 residue. The N439K mutation has also exhibited immune escape from polyclonal sera from a proportion of recovered individuals and some neutralizing mAbs (15).

References:

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