

DESCRIPTION

Source Human embryonic kidney cell, HEK293-derived sars-cov-2 Spike RBD protein
Arg319-Phe541 (Leu455Phe), with a C-terminal 6-His tag
Accession # YP_009724390.1

N-terminal Sequence Analysis Arg319

Predicted Molecular Mass 26 kDa

SPECIFICATIONS

Size Exclusion Chromatography 31-38 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.

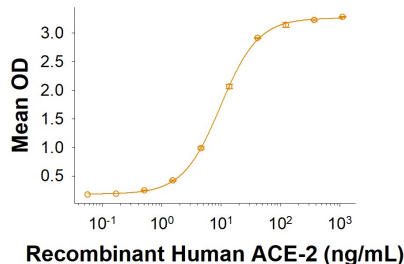
Shipping The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

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.

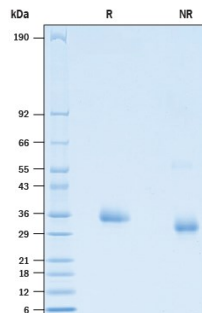
DATA

Binding Activity



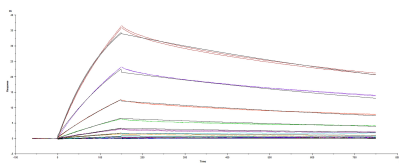
Recombinant SARS-CoV-2 L455F Spike RBD His-tag Protein Binding Activity.
Recombinant SARS-CoV-2 L455F Spike RBD His-tag (Catalog # 10817-CV) binds Recombinant Human ACE-2 His-tag (Catalog # 933-ZN) in a functional ELISA.

SDS-PAGE



Recombinant SARS-CoV-2 L455F Spike RBD His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant SARS-CoV-2 L455F Spike RBD His-tag Protein (Catalog # 10817-CV) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 31-38 kDa.

Surface Plasmon Resonance (SPR)



Binding of ACE-2 to SARS-CoV-2 Spike RBD protein with L455F mutation by surface plasmon resonance (SPR).
Recombinant SARS-CoV-2 Spike RBD protein L455F 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 0.046 nM and 47.2 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 KD= 5.24 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 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 metalloproteinase, angiotensin-converting enzyme 2 (ACE-2), has been identified as a functional receptor for SARS-CoV-2 through interaction with a receptor binding domain (RBD) located at the C-terminus of S1 subunit (6,7). 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. SARS-CoV-2 variants carrying the aa substitution L455F in the RBD was reported to escape neutralization of antibodies (8).

References:

1. Wu, F. *et al.* (2020) *Nature* **579**:265.
2. Tortorici, M.A. and D. Veesele (2019) *Adv. Virus Res.* **105**:93.
3. Bosch, B.J. *et al.* (2003) *J. Virol.* **77**:8801.
4. Belouzard, S. *et al.* (2009) *Proc. Natl. Acad. Sci.* **106**:5871.
5. Millet, J.K. and G.R. Whittaker (2015) *Virus Res.* **202**:120.
6. Li, W. *et al.* (2003) *Nature* **426**:450.
7. Wong, S.K. *et al.* (2004) *J. Biol. Chem.* **279**:3197.
8. Weisblum, Y. *et al.* (2020) *eLife* **9**:e61312.