

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived sars-cov-2 Spike protein		
	SARS-CoV-2 Spike (Val16-Lys1211)(Asp80Ala, Asp215Gly, Leu242del, Ala243del, Leu244del, Lys417Asn, Glu484Lys, Asn501Tyr, Asp614Gly, Ala701Val)(Arg682Ser, Arg685Ser, Lys986Pro, Val987Pro) Accession # YP_009724390.1	GCN4-IZ	6-His tag
	N-terminus		C-terminus
N-terminal Sequence Analysis	Val16		
Predicted Molecular Mass	138 kDa		

SPECIFICATIONS

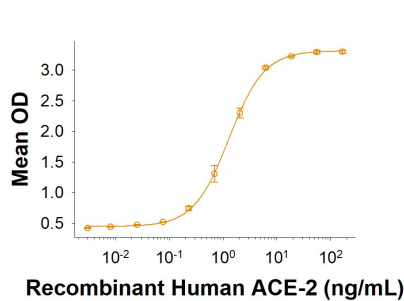
SDS-PAGE	145-168 kDa under reducing conditions.
Activity	Measured by its binding ability in a functional ELISA with Recombinant Human ACE-2 His-tag (Catalog # 933-ZN).
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
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	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> • 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.

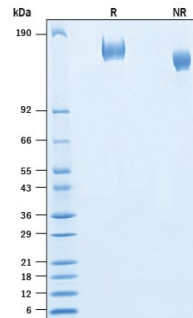
DATA

Binding Activity



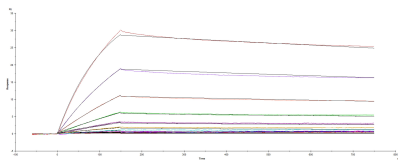
Recombinant SARS-CoV-2 B.1.351 Spike GCN4-IZ His-tag Protein Binding Activity. Recombinant SARS-CoV-2 B.1.351 Spike (GCN4-IZ) His-tag (Catalog # 10786-CV) binds Recombinant Human ACE-2 His-tag (Catalog # 933-ZN) in a functional ELISA.

SDS-PAGE



Recombinant SARS-CoV-2 B.1.351 GCN4-IZ His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant SARS-CoV-2 B.1.351 Spike GCN4-IZ His-tag (Catalog # 10786-CV) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 145-168 kDa.

Surface Plasmon Resonance (SPR)



Binding of ACE-2 to SARS-CoV-2 B.1.351 variant Spike protein by surface plasmon resonance (SPR). Recombinant SARS-CoV-2-B.1.351 South Africa variant Spike protein 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 $K_D=1.272$ 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 are commonly comprised of four structural proteins: Spike protein (S), Envelope protein (E), Membrane protein (M), and Nucleocapsid protein (N) (1). SARS-CoV-2 Spike Protein (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 the 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). The S protein of SARS-CoV-2 shares 75% and 29% amino acid (aa) sequence identity with the S protein of SARS-CoV-1 and MERS, respectively. The S Protein of the SARS-CoV-2 virus, like the SARS-CoV-1 counterpart, binds Angiotensin-Converting Enzyme 2 (ACE-2), but with much higher affinity and faster binding kinetics through the receptor binding domain (RBD) located in the C-terminal region of S1 (6). Based on structural biology studies, the RBD can be oriented either in the up/standing or down/lying state with the up/standing state associated with higher pathogenicity (7). 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 (8). It has been demonstrated that the S Protein can invade host cells through the CD147/EMMPRIN receptor and mediate membrane fusion (9, 10). A SARS-CoV-2 variant carrying amino acid substitutions N501Y, K417N, and E484K in the RBD raised the most concerns. This B.1.351 lineage, also known as 501Y.V2 variant, was first identified in the Eastern Cape province of South Africa in December 2020 and spread quickly to become the most dominant strain in the second COVID wave in South Africa (11). Two of these mutations K417N and E484K locate at the receptor binding motif (RBM) and are not found in other variants (11). The N501Y mutation is also found in London (B.1.1.7 lineage) and Brazil (P.1 lineage). The B.1.351 lineage is reported to enter cells more easily due to its enhanced affinity to ACE-2 receptor (12). It is reported to reduce the efficacy of neutralizing antibody (12, 13).

References:

1. Wu, F. *et al.* (2020) *Nature* **579**:265.
2. Tortorici, M.A. and D. Veesler (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. Ortega, J.T. *et al.* (2020) *EXCLI J.* **19**:410.
7. Yuan, Y. *et al.* (2017) *Nat. Commun.* **8**:15092.
8. Tai, W. *et al.* (2020) *Cell. Mol. Immunol.* <https://doi.org/10.1016/j.cmi.2020.03.007>.
9. Wang, X. *et al.* (2020) <https://doi.org/10.1038/s41423-020-0424-9>.
10. Wang, K. *et al.* (2020) *bioRxiv* <https://doi.org/10.1101/2020.03.14.988345>.
11. Tegally, H. *et al.* (2020) *bioRxiv*. Doi: <https://doi.org/10.1101/2020.12.21.20248640>.
12. Nelson, G. *et al.* (2021) *bioRxiv*. <https://doi.org/10.1101/2021.01.13.426558>.
13. Wibmer, C.K. *et al.* (2021) *bioRxiv*. <https://doi.org/10.1101/2021.01.18.427166>.