R&D SYSTEMS

Recombinant SARS-CoV-2 D614G Spike S1 Subunit His-tag

Catalog Number: 10609-CV

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived sars-cov-2 Spike S1 Subunit protein Val16-Pro681 (Asp614Gly), with a C-terminal 6-His tag Accession # YP_009724390.1
N-terminal Sequence Analysis	Val16
Predicted Molecular Mass	75 kDa

SPECIFICATIONS

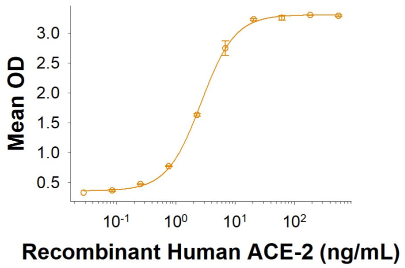
SDS-PAGE	102-116 kDa, under reducing conditions
Activity	Recombinant SARS-CoV-2 D614G Spike S1 Subunit His-tag (Catalog # 10609-CV) binds Recombinant Human ACE-2 His-tag (Catalog # 933-ZN) in a functional ELISA.
Endotoxin Level	<0.10 EU per 1 μg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE 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.

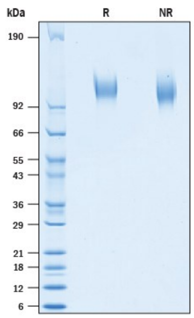
DATA

Binding Activity



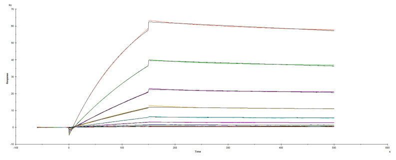
Recombinant SARS-CoV-2 D614G Spike S1 Subunit His-tag Protein Binding Activity. Recombinant SARS-CoV-2 D614G Spike S1 Subunit His-tag (Catalog # 10609-CV) binds Recombinant Human ACE-2 His-tag (Catalog # 933-ZN) in a functional ELISA.

SDS-PAGE



Recombinant SARS-CoV-2 D614G Spike S1 Subunit His-tag Protein SDS-PAGE. 2 μg/lane of Recombinant SARS-CoV-2 D614G Spike S1 Subunit His-tag Protein (Catalog # 10609-CV) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 95-120 kDa.

Surface Plasmon Resonance (SPR)



Binding of ACE-2 to SARS-CoV-2 Spike S1 Subunit D614G by surface plasmon resonance (SPR). Recombinant SARS-CoV-2 Spike S1 Subunit D614G 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 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=1.546 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 homotrimeric glycoprotein that mediates membrane fusion and viral entry. As with most coronaviruses, proteolytic cleavage of the SARS-CoV-2 S protein into two distinct peptides, 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 (2-5). A SARS-CoV-2 variant carrying the S protein amino acid (aa) change D614G has become the most prevalent form in the global pandemic and has been associated with greater infectivity and higher viral load (6,7). Based on structural biology studies, the receptor binding domain (RBD), located in the C-terminal region of S1, can be oriented either in the up/standing or down/lying state (8). The standing state is associated with higher pathogenicity and both SARS-CoV-1 and MERS can access this state due to the flexibility in their respective RBDs. A similar two-state structure and flexibility is found in the SARS-CoV-2 RBD (9). Based on amino acid (aa) sequence homology, the SARS-CoV-2 S1 subunit has 65% identity with SARS-CoV-1 S1 subunit, but only 22% homology with the MERS S1 subunit. The low aa sequence homology is consistent with the finding that SARS and MERS bind different cellular receptors (10). 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 (11). Before binding to the ACE-2 receptor, structural analysis of the S1 trimer shows that only one of the three RBD domains in the trimeric structure 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 (12). Polyclonal antibodies to the RBD of the SARS-CoV-2 S1 subunit have been shown to inhibit interaction with the ACE-2 receptor, confirming S1 subunit especially the RBD as an attractive target for vaccinations or antiviral therapy (13). 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 virus (14). Lastly, it has been demonstrated the S Protein can invade host cells through the CD147/EMMPRIN receptor and mediate membrane fusion (15, 16).

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. Korber, *et al.* (2020) Cell 182, 812.
7. Zhang, L. *et al.* (2020) ioRxiv https://www.biorxiv.org/content/10.1101/2020.06.12.148726v1.
8. Yuan, Y. *et al.* (2017) Nat. Commun. **8**:15092.
9. Walls, A. C. *et al.* (2010) Cell **180**:281.
10. Jiang, S. *et al.* (2020) Trends. Immunol. https://doi.org/10.1016/j.it.2020.03.007.
11. Ortega, J. T. *et al.* (2020) EXCLI J. **19**:410.
12. Wrapp, D. *et al.* (2020) Science **367**:1260.
13. Tai, W. *et al.* (2020) Cell. Mol. Immunol. https://doi.org/10.1016/j.it.2020.03.007.
14. Okba, N. M. A. *et al.* (2020). Emerg. Infect. Dis. https://doi.org/10.3201/eid2607.200841.
15. Wang, X. *et al.* (2020) https://doi.org/10.1038/s41423-020-0424-9.
16. Wang, K. *et al.* (2020) ioRxiv https://www.biorxiv.org/content/10.1101/2020.03.14.988345v1.