Recombinant SARS-CoV-2 B.1.1.7 Spike His-tag

Catalog Number: 10748-CV

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived sars-cov-2 Spike protein Val16-Lys1211 (His69 del, Val70 del, Tyr145 del, Asn501Tyr, Ala570Asp, Asp614Gly, Pro681His, Thr716Ile, Ser982Ala, Asp1118His) (Arg682Ser, Arg685Ser, Lys986Pro, Val987Pro), with a C-terminal 6-His tag Accession # YP_009724390.1
N-terminal Sequence Analysis	Protein identity is confirmed by mass spectrometry.
Predicted Molecular Mass	134 kDa

SPECIFICATIONS

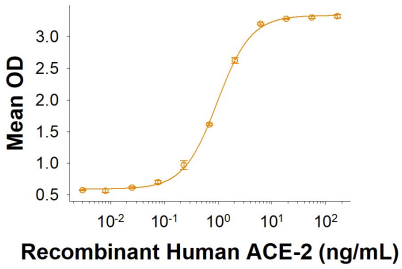
SDS-PAGE	148-166 kDa, under reducing conditions
Activity	Measured by its binding ability in a functional ELISA with Recombinant Human ACE-2 His-tag (Catalog # 933-ZN).
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative 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.

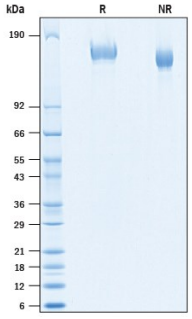
DATA

Binding Activity



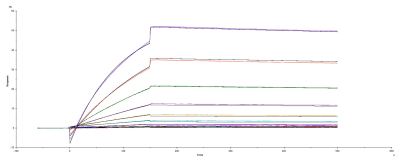
Recombinant SARS-CoV-2 B.1.1.7 Spike His-tag Protein Binding Activity. Recombinant SARS-CoV-2 B.1.1.7 Spike His-tag (Catalog # 10748-CV) binds Recombinant Human ACE-2 His-tag (Catalog # 933-ZN) in a functional ELISA.

SDS-PAGE



Recombinant SARS-CoV-2 B.1.1.7 Spike His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant SARS-CoV-2 B.1.1.7 Spike His-tag (Catalog # 10748-CV) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 148-166 kDa.

Surface Plasmon Resonance (SPR)



Binding of ACE-2 to UK variant B.1.1.7 Spike protein by surface plasmon resonance (SPR). Recombinant SARS-CoV-2 Spike protein UK variant B.1.1.7 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 KD=1.185 nM.

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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 (ACE2), 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 ACE2 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 the aa substitution N501Y in the RBD and nine additional mutations in the rest of the spike protein becomes one of the most prevalent mutations found in Covid-19 cases (11-13). This mutant (B.1.1.7 lineage) was originally found in London and the southeast UK but rapidly spread globally (8,9). This new virus variant was reported 56% more transmissible than other preexisting variants (14). The N501Y mutation was also later identified in variants found in South Africa (B.1.351 lineage) and Brazil (P.1 lineage). Although there is no evidence to date that B.1.1.7 causes more severe illness, whether the N501Y mutation in RBD would decrease the efficacy of vaccine-induced immunity is still under investigation.

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