Recombinant SARS-CoV-2 Spike His-tag

Catalog Number: 10549-CV

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived sars-cov-2 Spike protein Val16-Lys1211 (Arg682Ser, Arg685Ser, Lys986Pro and Val987Pro) with a C-terminal 6-His tag Accession # YP_009724390.1 Suitable for use in serological assay development
N-terminal Sequence Analysis	Val16
Predicted Molecular Mass	134 kDa

SPECIFICATIONS

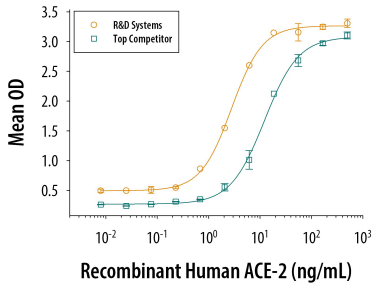
SDS-PAGE	144-175 kDa, under reducing conditions
Activity	Measured by its binding ability in a functional ELISA with Recombinant Human ACE-2 His-tag (Catalog # 933-ZN).
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative 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.

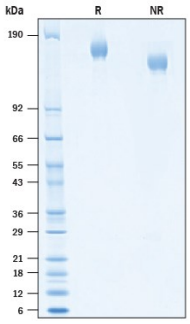
DATA

Bioactivity



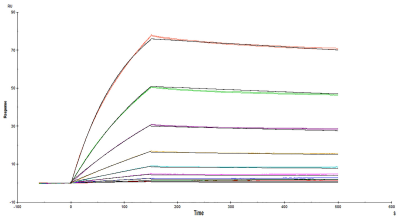
Recombinant SARS-CoV-2 Spike His Protein Binding Activity R&D Systems Recombinant SARS-CoV-2 Spike (Catalog # 10549-CV) binds Recombinant Human ACE-2 (Catalog # 933-ZN) in a functional ELISA. The binding activity is approximately 4-fold greater than a top competitor's Spike protein (full ectodomain).

SDS-PAGE



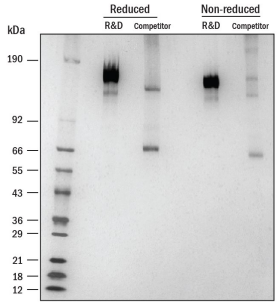
Recombinant SARS-CoV-2 Spike His Protein SDS-PAGE 2 µg/lane of Recombinant SARS-CoV-2 Spike His Protein (Catalog # 10549-CV) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 144-175 kDa.

Surface Plasmon Resonance (SPR)



Binding of ACE-2 to SARS-CoV-2 Spike by surface plasmon resonance (SPR). Recombinant SARS-CoV-2 Spike His Protein (Catalog # 10549-CV) was immobilized on a Biacore Sensor Chip CM5, and binding to recombinant human ACE-2 (Catalog # Catalog # 933-ZN) was measured at a concentration range between 0.37 nM and 93.5 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=1.720 nM. (Biacore T200).

SDS-PAGE



Recombinant SARS-CoV-2 Spike His Protein SDS-PAGE SDS-PAGE of 1 µg of R&D Systems Recombinant SARS-CoV-2 Spike (Catalog # 10549-CV) or a competitor's Spike protein were run under reducing or non-reducing conditions and visualized by silver staining. The R&D Systems Spike protein runs as a single band compared to the competition.

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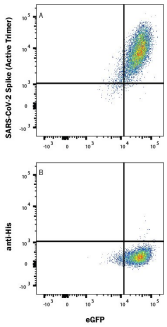
Flow Cytometry



Detection of SARS-CoV-2 Spike Protein bound to ACE-2 expressing cells by flow cytometry. In a functional flow cytometry test, (A) Recombinant SARS-CoV-2 Spike His-tag Protein (Catalog # 10549-CV) binds to HEK293 human embryonic kidney cell line transfected with recombinant human ACE-2 and EGFP. Ligand binding was detected by staining cells with APC-conjugated anti-His Monoclonal Antibody (Catalog # IC050A), which does not stain the cells in the absence of recombinant protein (B).

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 S protein aa change D614G has become the most prevalent form in the global pandemic and has been associated with greater infectivity and higher viral load (11, 12).

References:

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