Recombinant SARS-CoV-2 Spike RBD Fc Chimera

Catalog Number: 10499-CV

R&D SYSTEMS a bio-techne brand

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived sars-cov-2 Spike RBD protein

SARS-CoV2 Spike RBD (Arg319-Phe541) Accession # YP_009724390.1	IEGRMD	Human IgG_1 (Pro100-Lys330)
N-terminus		C-terminus

N-terminal Sequence Analysis	Arg319
Structure / Form	Disulfide-linked homodimer
Predicted Molecular Mass	52 kDa

SPECIFICATIONS

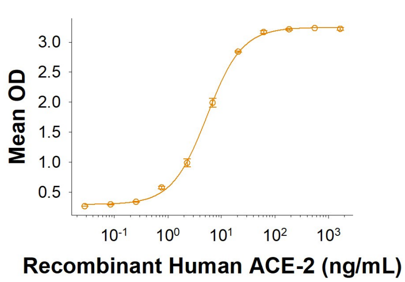
SDS-PAGE	55-66 kDa, under reducing conditions
Activity	Measured by its binding ability in a functional ELISA with Recombinant Human ACE-2 His-tag (Catalog # 933-ZN). Measured by its binding ability in a functional ELISA with Recombinant Human EMMPRIN/CD147 Fc Chimera (Catalog # 972-EMN).
Endotoxin Level	<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.

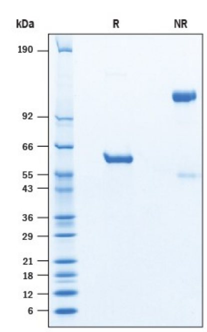
DATA

Bioactivity



Recombinant SARS-CoV-2 Spike RBD Fc Chimera Protein (Catalog # 10499-CV) binds Recombinant Human ACE-2 His-tag (Catalog # 933-ZN) in a functional ELISA.

SDS-PAGE



2 µg/lane of Recombinant SARS-CoV-2 Spike RBD Fc Chimera Protein (Catalog # 10499-CV) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 55-66 kDa and 110-132 kDa, respectively.

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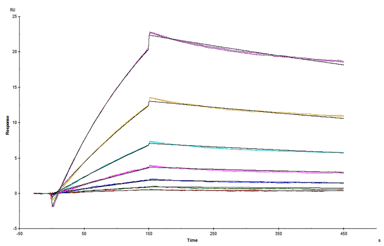
Surface Plasmon Resonance (SPR)



Recombinant SARS-CoV-2 Spike RBD Fc Chimera Protein binds to Recombinant Human ACE-2 by surface plasmon resonance Recombinant SARS-Cov-2 Spike RBD Fc Chimera Protein (Catalog # 10499-CV) 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 0.73 nM and 46.7 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=7.037 nM. (Biacore T200).

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 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 (3-5). 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 (6). 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 (7). Based on amino acid (aa) sequence homology, the SARS-CoV-2 S1 subunit RBD has 73% identity with the RBD of the SARS-CoV-1 S1 RBD, but only 22% homology with the MERS S1 RBD. The low aa sequence homology is consistent with the finding that SARS and MERS bind different cellular receptors (8). 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 (9). Before binding to the ACE2 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 (10). 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 (11). 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 (12). Lastly, it has been demonstrated the S Protein can invade host cells through the CD147/EMMPRIN receptor and mediate membrane fusion (13, 14).

References:

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