SARS-CoV-1/2 Spike RBD Llamabody™ Antibody

Recombinant Monoclonal Human IgG_1 Clone # VHH72

Catalog Number: LMAB10541

DESCRIPTION

Species Reactivity	SARS-CoV-1/2
Specificity	Detects Recombinant SARS-CoV-2 Spike Protein S1 Receptor Binding Domain (RBD) in direct ELISA. In direct ELISA, no cross-reactivity with SARS-CoV-2 Spike Protein S2 is observed. VHH72 binding to SARS-CoV-1 RBD and SARS-CoV-2 has been reported in Wrapp, D. *et al.* (2020) Cell **181**: 1004.
Source	Recombinant Monoclonal Human IgG_1 Clone # VHH72
Purification	Protein A or G purified from cell culture supernatant
Immunogen	Recombinant Llama VHH sequence based on VHH72 in Wrapp, D. *et al.* (2020) Cell **181**: 1004. Bivalent Llama VHH-Human IgG1 Fusion Antibody. Accession # YP_009724390
Endotoxin Level	<1.0 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.

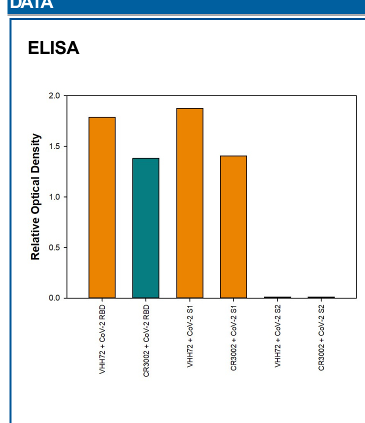
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

Blockade of Receptor-ligand Interaction	In a functional flow cytometry test, 25 µg/mL of Anti-SARS CoV-1/2 Spike RBD Llamabody blocks the binding of His-tagged recombinant SARS-CoV-2 Spike RBD (50 ng/mL, Catalog # 10534-CV) to HEK293 human embryonic kidney cell line transfected with human ACE-2.
ELISA	Detects SARS-CoV-2 Spike Protein S1 and SARS CoV-2 Spike Protein RBD in direct ELISA.

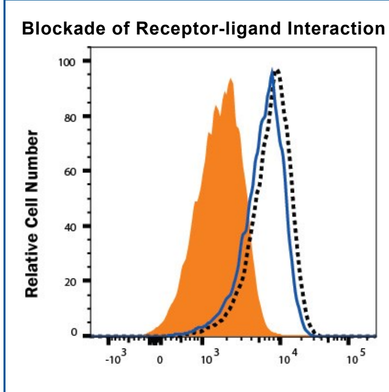
DATA

ELISA



Specificity of Anti-SARS-CoV-1/2 Spike RBD (Clone VHH72) Llamabody in Direct ELISA. A panel of SAR-CoV-2 Spike and RBD proteins were coated at 2 µg/mL in 96-well polystyrene plates. Anti-SARS-CoV-1/2 RBD (Clone VHH72) Llamabody (Catalog # LMAB10541, 7.81 ng/mL) detected SARS-CoV-2 Spike Protein S1 and SARS CoV-2 Spike Protein RBD. Anti-SARS RBD Clone CR3022 was included as a positive control for detection of SARS-CoV-2 Spike Protein S1 and SARS CoV-2 Spike Protein RBD. No cross-reactivity with SARS-CoV-2 Spike Protein S2 was detected.

Blockade of Receptor-ligand Interaction



SARS-CoV-2 Spike RBD Binding to ACE-2-transfected HEK293 Human Cell Line is Blocked by SARS-Cov-1/2 Spike RBD Llamabody. In a functional flow cytometry test, His-tagged recombinant SARS-CoV-2 Spike RBD (50 ng/mL, Catalog # 10534-CV) binds to HEK293 human embryonic kidney cell line transfected with human ACE-2 (black dotted line). Binding is completely blocked (orange histogram) by 25 µg/mL of Anti-SARS CoV-1/2 S1 RBD Llamabody (Catalog # LMAB10541). Mouse anti-Human DC-SIGN Monoclonal Antibody (Catalog # MAB161) at 25 µg/mL was used as an irrelevant control (blue line). Cells were stained with Allophycocyanin-conjugated Mouse anti-His tag Monoclonal Antibody (Catalog # IC050A).

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile 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. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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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