

DESCRIPTION

Species Reactivity	SARS-CoV-2
Specificity	Detects SARS-CoV2 SARS-CoV2 spike in direct ELISA.
Source	Monoclonal Mouse IgG _{2A} Clone # 1049621
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Human embryonic kidney cell, HEK293-derived SARS-CoV2 SARS-CoV2 spike Arg319-Phe541 Accession # P0DTC2
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

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.

	Recommended Concentration	Sample
Immunocytochemistry	8-25 µg/mL	Immersion fixed Transfected & Wild Type HEK293 Human Embryonic Kidney Cell Line
Neutralization	In a functional ELISA, 0.0800 - 1.20 µg/mL of this antibody will block 50% of the binding of 50 ng/mL Recombinant Human ACE-2 (Catalog # 933-ZN) to Recombinant SARS-CoV-2 B.1.525 Spike immobilized at 0.5 ug/mL (100 µL/well).	
Blockade of Receptor-ligand Interaction	In a functional flow cytometry test, 25 µg/mL of Mouse Anti-SARS2-B.1.525S Antibody (Catalog # MAB11292) will block the binding Recombinant SARS-CoV2-B.1.525S Protein to HEK293 human embryonic kidney cell line transfected with recombinant human ACE-2.	

DATA

Immunocytochemistry

Detection of Spike RBD in Transfected & Wild Type HEK293 Cell Line. Spike RBD was detected in immersion fixed Transfected & absent in Wild Type HEK293 Human Embryonic Kidney Cell Line using Mouse Anti-SARS-CoV-2 Spike RBD Monoclonal Antibody (Catalog # MAB11292) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cell surface and cytoplasm. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Blockade of Receptor-ligand Interaction

SARS-CoV-2 variant protein (named B.1.525S) binding to ACE-2-transfected Human Cell Line is Blocked by SARS2-B.1.525S Antibody. In a functional flow cytometry test, Recombinant SARS-CoV2-B.1.525S His-tagged protein binds to HEK293 human embryonic kidney cell line transfected with recombinant human ACE-2 and eGFP. (A) Binding is completely blocked by 25 µg/mL of Mouse Anti-SARS2-B.1.525S Antibody (Catalog #MAB11292) but not by (B) Mouse IgG2A Isotype Control (Catalog # MAB003). Protein binding was detected with Mouse Anti-His APC-conjugated Monoclonal Antibody (Catalog # IC050A). Staining was performed using our [Staining Membrane-associated Proteins](#) protocol.

Neutralization

SARS-CoV-2 B.1.525 Spike RBD Antibody (µg/mL)

ACE-2 Binding to SARS-CoV-2 B.1.525 Spike is Blocked by SARS-CoV-2 Spike RBD Antibody. In a functional ELISA, 0.0800 - 1.20 µg/mL of this antibody will block 50% of the binding of 50 ng/mL Recombinant Human ACE-2 (Catalog # 933-ZN) to Recombinant SARS-CoV-2 B.1.525 Spike immobilized at 0.5 ug/mL (100 µL/well).

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. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> • 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.

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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