

DESCRIPTION

Species Reactivity	SARS-CoV-2
Specificity	Detects Sars-cov-2 Spike RBD protein in Elisas and Western Blot
Source	Recombinant Monoclonal Rabbit IgG Clone # 2847A
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	CHO-derived sars-cov-2 Spike RBD protein Arg319-Phe541 Accession # YP_009724390.1
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.

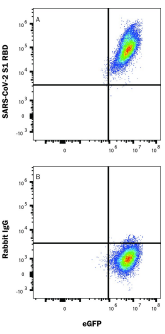
APPLICATIONS

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
Western Blot	2 µg/mL	rSARS2-S1 RBD (DNVY28)
Flow Cytometry	0.25 µg/10 ⁶ cells	HEK293 human embryonic kidney cell line transfected with human ACE-2 and eGFP incubated with Recombinant SARS-CoV-2 Spike S1 Subunit His-Tag protein (Catalog # 10534-CV)

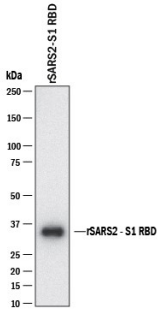
DATA

Flow Cytometry



Detection of SARS-CoV-2 Spike S1 protein bound to ACE-2 in HEK293 Human Cell Line Transfected with Human ACE-2 and eGFP by Flow Cytometry. HEK293 human embryonic kidney cell line transfected with human ACE-2 and eGFP was incubated with Recombinant SARS-CoV-2 Spike S1 Subunit His-Tag protein (Catalog # 10534-CV), then stained with (A) Rabbit Anti-SARS-CoV-2 Spike S1 Monoclonal Antibody (Catalog # MAB11198) or (B) Rabbit IgG Isotype Control Antibody (Catalog # MAB1050) followed by Allophycocyanin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0111). Staining was performed using our Staining Membrane-associated Proteins protocol.

Western Blot



Detection of SARS-CoV-2 Spike RBD by Western Blot. Western blot shows lysates of rSARS2-S1 RBD (DNVY28). PVDF membrane was probed with 2 µg/mL of Rabbit Anti-SARS-CoV-2 Spike RBD Monoclonal Antibody (Catalog # MAB11198) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for Spike RBD at approximately 35 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

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	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <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 (ACE-2), but with much higher affinity and faster binding kinetics (9). Before binding to the ACE-2 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 ACE-2 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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