

## DESCRIPTION

<b>Species Reactivity</b>	SARS-CoV-2
<b>Specificity</b>	Detects human SARS-CoV-2 spike S2 in direct ELISAs and Western Blot
<b>Source</b>	Recombinant Monoclonal Rabbit IgG Clone # 2824E
<b>Purification</b>	Protein A or G purified from cell culture supernatant
<b>Immunogen</b>	Chinese Hamster Ovary cell line, CHO-derived human SARS-CoV-2 Spike protein Met1-Lys1211 Accession # YP_009724390.1
<b>Formulation</b>	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.

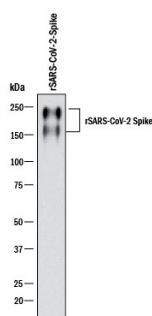
## APPLICATIONS

**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
<b>Western Blot</b>	1 µg/mL	Recombinant SARS-CoV-2 spike protein
<b>Immunocytochemistry</b>	3-25 µg/mL	Immersion fixed HEK293 human embryonic kidney cell line transfected
<b>Immunohistochemistry</b>	3-25 µg/mL	Immersion fixed paraffin-embedded sections of SARS-CoV-2 infected human lung

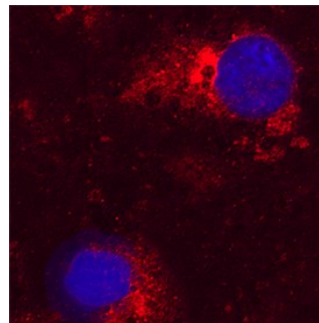
## DATA

### Western Blot



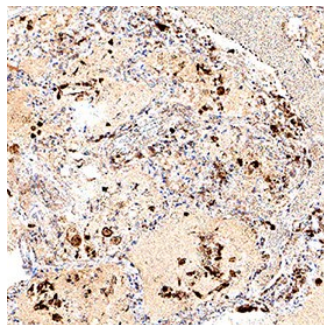
**Detection of SARS-CoV-2 Spike Protein by Western Blot.** Western blot shows recombinant SARS-CoV-2 spike protein. PVDF membrane was probed with 1 µg/mL of Rabbit Anti-SARS-CoV-2 Spike S2 Subunit Monoclonal Antibody (Catalog # MAB108891) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for the Spike Protein at approximately 150-250 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

### Immunocytochemistry



**Spike S2 Subunit in HEK293 Human Cell Line.** Spike S2 Subunit was detected in immersion fixed HEK293 human embryonic kidney cell line transfected using Rabbit Anti-SARS-CoV-2 Spike S2 Subunit Monoclonal Antibody (Catalog # MAB108891) at 3 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. Staining was performed using our protocol for Fluorescent ICC Staining of Non-adherent Cells.

### Immunohistochemistry



**Spike S2 Subunit in SARS-CoV-2 infected human lung.** Spike S2 Subunit was detected in immersion fixed paraffin-embedded sections of SARS-CoV-2 infected human lung using Rabbit Anti-SARS-CoV-2 Spike S2 Subunit Monoclonal Antibody (Catalog # MAB108891) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to immunoreactive profiles scattered throughout the tissue. Staining was performed using our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

## PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.5 mg/mL in sterile PBS.
<b>Shipping</b>	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
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>• 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

## 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 has 65% identity with SARS-CoV-1 S1 subunit, but only 22% homology with the MERS S1 subunit. 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 S1 subunit 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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