

DESCRIPTION

Species Reactivity	SARS-CoV-2
Specificity	Detects SARS-CoV-2 Spike S2 Subunit in direct ELISAs.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Human embryonic kidney cell HEK293-derived SARS-CoV-2 Spike S2 Subunit Ser686-Lys1211 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.

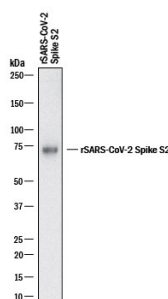
APPLICATIONS

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	1 µg/mL	Recombinant SARS-CoV-2 Spike S2
Immunocytochemistry	8-25 µg/mL	Immersion fixed HEK293 human embryonic kidney cell line transfected with SARS-CoV-2
Immunohistochemistry	1-25 µg/mL	Immersion fixed paraffin-embedded sections of SARS-CoV-2 infected human lung

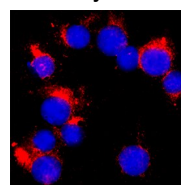
DATA

Western Blot

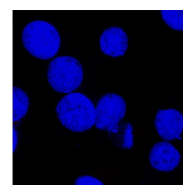


Detection of SARS-CoV-2 Spike S2 Subunit by Western Blot. Western blot shows recombinant SARS-CoV-2 Spike S2. PVDF membrane was probed with 1 µg/mL of Goat Anti-SARS-CoV-2 Spike S2 Subunit Antigen Affinity-purified Polyclonal Antibody (Catalog # AF10774) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for Spike S2 Subunit at approximately 67 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

Immunocytochemistry



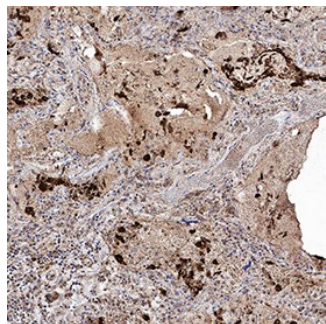
Positive (HEK293 transfected cells)



Negative (HEK293 cells)

Spike S2 Subunit in HEK293 human embryonic kidney cell line transfected with SARS-CoV-2. Spike S2 Subunit was detected in immersion fixed HEK293 human embryonic kidney cell line transfected with SARS-CoV-2 (positive staining) and HEK293 human embryonic kidney cell line (non-transfected, negative staining) using Goat Anti-SARS-CoV-2 Spike S2 Subunit Antigen Affinity-purified Polyclonal Antibody (Catalog # AF10774) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cell 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 Goat Anti-SARS-CoV-2 Spike S2 Subunit Antigen Affinity-purified Polyclonal Antibody (Catalog # AF10774) at 1 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). 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

Reconstitution	Reconstitute at 0.2 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 also include MERS and SARS-CoV-1. Coronaviruses 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). 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 (2-4). A metalloprotease, angiotensin-converting enzyme 2 (ACE-2), has been identified as a functional receptor for SARS-CoV2, similar to SARS-CoV-1, through interaction with a receptor binding domain (RBD) located at the C-terminus of S1 subunit (5, 6). The S2 subunit of SARS-CoV-2 shares 90% and 41% amino acid sequence identity with the S2 subunit of SARS-CoV-1 and MERS, respectively. It has been demonstrated the S Protein can invade host cells through the CD147/EMMPRIN receptor and mediate membrane fusion (7, 8).

References:

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3. Belouzard, S. *et al.* (2009) *Proc. Natl. Acad. Sci. USA* **106**:5871.
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5. Li, W. *et al.* (2003) *Nature* **426**:450.
6. Wong, S.K. *et al.* (2004) *J. Biol. Chem.* **279**:3197.
7. Wang, X. *et al.* (2020) <https://doi.org/10.1038/s41423-020-0424-9>.
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