DESCRIPTION

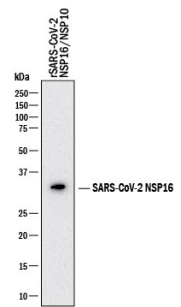|	
Species Reactivity	SARS-CoV-2
Specificity	Detects SARS-CoV-2 NSP16/NSP10 in direct ELISAs
Source	Monoclonal Mouse IgG$_{2B}$ Clone # 1044311
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived SARS-CoV-2 NSP16/NSP10 SARS-CoV-2 NSP16 (Ser1-Asn298) and SARS-CoV-2 NSP10 (Ala1-Gln139) Accession # YP_009725311.1 and YP_009725306.1
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.*

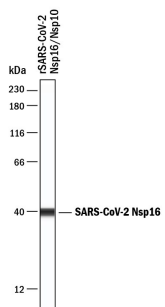|	Recommended Concentration	Sample
Western Blot	1 µg/mL	Recombinant SARS-CoV-2 NSP16/NSP10
Simple Western	20 µg/mL	Recombinant SARS-CoV-2 Nsp10/Nsp16
ELISA		This antibody functions as an ELISA detection antibody when paired with Mouse Anti-SARS-CoV-2 NSP16 Monoclonal Antibody (Catalog # MAB10941).This product is intended for assay development on various assay platforms requiring antibody pairs.

DATA

Western Blot

Detection of SARS-CoV-2 NSP16 by Western Blot.
Western blot shows recombinant SARS-CoV-2 NSP16/NSP10. PVDF membrane was probed with 1 µg/mL of Mouse Anti-SARS-CoV-2 NSP16 Monoclonal Antibody (Catalog # MAB10940) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for NSP16/NSP10 at approximately 34 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.
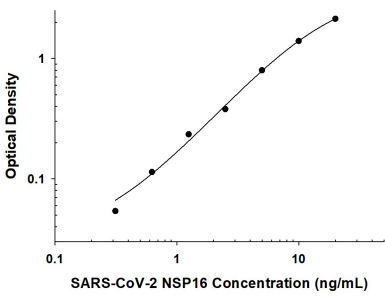
Simple Western

Detection of SARS-CoV-2 NSP16 by Simple Western™.
Simple Western lane view shows recombinant SARS-CoV-2 Nsp10/Nsp16, loaded at 0.2 mg/mL. A specific band was detected for NSP16 at approximately 39 kDa (as indicated) using 20 µg/mL of Mouse Anti-SARS-CoV-2 NSP16 Monoclonal Antibody (Catalog # MAB10940) . This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

ELISA

SARS-CoV-2 NSP16/NSP10 ELISA Standard Curve.
Recombinant SARS-CoV-2 NSP16/NSP10 protein was serially diluted 2-fold and captured by Mouse Anti-SARS-CoV-2 His-tag Complex Monoclonal Antibody (Catalog # MAB10941) coated on a Clear Polystyrene Microplate (Catalog # DY990). Mouse Anti-SARS-CoV-2 NSP16 Monoclonal Antibody (Catalog # MAB10940) was biotinylated and incubated with the protein captured on the plate. Detection of the standard curve was achieved by incubating Streptavidin-HRP (Catalog # DY998) followed by Substrate Solution (Catalog # DY999) and stopping the enzymatic reaction with Stop Solution (Catalog # DY994).

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.** • 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

Non-structural protein 16 (NSP16) and NSP10 are two of several functional proteins released by ORF1a-encoded protease cleavage of the pp1a and pp1ab replicase polyproteins expressed from the coronavirus (CoV) genome (1). The NSPs are involved in the replication and transcription of the viral RNA and not incorporated within the virion particles. Coronaviruses include various highly pathogenic strains such as SARS-CoV, MERS-CoV and SARS-CoV2 that have had significant impact on humans as well as strains that have negatively impacted livestock. NSP16 is a small monomeric 298 amino acid protein with a characteristic fold of the class I methyltransferase (MTase) family with a seven-stranded B-sheet surrounded by alpha-helices and loops that bind the cap and form a cap-binding groove and S-adenosylmethionine (SAM) cleft (2, 3). NSP10 is a small 139 amino acid protein capable of forming independent dodecameric structures composed of four identical trimers where each monomer is capable of binding two zinc ions (4, 5). Both the NSP16 and NSP10 SARS-CoV2 sequences are highly conserved across coronaviruses (3). NSP16 catalyzes 2'-O-MTase activity specifically on capped N7-methylated RNA through methylation of the 2'-hydroxy group of adenine using SAM as a methyl donor (6). The viral RNA cap structure protects it from degradation, promotes mRNA translation, and prevents the viral RNA from being recognized by innate immune mechanisms (7). NSP16 was shown to interact strongly with NSP10 (8) in a monomeric form via hydrophic interactions and hydrogen bonds in the interface (3). The MTase activity of NSP16 requires interaction with NSP10 (2, 7). NSP10 interaction extends and narrows the RNA-binding groove that accommodates the substrate and stabilizes NSP16 (2, 3, 9). The dimerization interface between NSP16 and NSP10 is critical and can be used as a target to effectively reduce replication and pathogenesis (10, 11).

References:

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