CERTIFICATE OF ANALYSIS

Anti-SARS-CoV Nucleocapsid (N) Protein (RABBIT) Antibody - 200-401-A50

Code: 200-401-A50 **Size:** 500 µg **Lot #:** 46527

Product Description: Anti-SARS-CoV Nucleocapsid (N) Protein (RABBIT) Antibody - 200-401-A50

Concentration: 5.3 mg/mL

Physical State: Lyophilized

Label:	Unconjugated
Host:	Rabbit
Species Reactivity:	Virus
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Stabilizer:	None
Preservative:	0.01% (w/v) Sodium Azide
Reconstitution Buffer:	Restore with deionized water (or equivalent)
Reconstitution Volume:	100 µL
Expiration:	Expiration date is one (1) year from date of opening.
Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Synonyms:	rabbit anti-Sars Nucleocapsid Protein Antibody, rabbit anti-Sars-CoV Nucleocapsid (N) Protein Antibody, N antibody, N structural protein antibody, NC antibody, Nucleocapsid protein antibody, Nucleoprotein antibody, SARS coronavirus N protein antibody, SARS CoV antibody, SARSCoV antibody, Severe acute respiratory syndrome antibody, A50 Antibody, A50 SARS, COVID
Background:	The coronavirus nucleocapsid protein is the major structural component of virions that associates with genomic RNA to form a long, flexible, helical nucleocapsid. Sequence comparison of the N genes of five strains of the coronavirus mouse hepatitis virus suggests a three-domain structure for the nucleocapsid protein. Anti-SARS-CoV Nucleocapsid (N) Protein Antibody is useful for researchers interested in viral research.
Application Note:	This protein A purified antibody has been tested for use in ELISA, western blot, Immunohistochemistry, Immunofluorescence, and lateral flow. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 46 kDa in size corresponding to SARS Nucleocapsid (N) protein by western blotting in the appropriate cell lysate or extract. ELISA and lateral flow format has been used to detect virus in extracts from nasal and throat swabs and saliva. IF has been used to determine the presence or absence of virus entering cells especially when anti-viral drugs are applied. IHC studies have been performed on biopsies, included retrospective studies on cadaver tissues after formalin fixation and paraffin embedding, detecting the coronavirus in lung, liver, bile duct, and placenta tissue. Yet other studies have shown this antibody has the ability to neutralize the virus and thereby protect cells from the uptake of live virus. Others have demonstrated the utility of the antibody in flow cytometry studies.
Purity and Specificity:	This protein A purified antibody is directed against SARS Coronavirus Nucleocapsid (N) protein. The product was purified from monospecific antiserum by protein A affinity purification. BLAST analysis was used to suggest reactivity with related Coronavirus proteins. Cross reactivity with homologues from other sources has not been determined.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:50,000
IF Microscopy:	User Optimized
	1:2,000 - 1:10,000

Western Blot:

Immunogen: This protein A purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a purified recombinant protein corresponding to full length SARS Coronavirus Nucleocapsid protein. Lifesensors Inc. (www.lifesensors.com) prepared the Nucleocapsid protein as follows: SUMO-Nucleocapsid fusion was expressed in E.coli in LB medium and purified using Ni-NTA resin (Qiagen) affinity chromatography. After the fusion was cleaved by the SUMO Protease (LifeSensors), the SUMO tag and protease were subtracted from the nucleocapsid using MAC and the nucleocapsid was finally purified using Cation Exchange Chromatography with the Macro-Prep High S resin (BioRad) and size exclusion chromatography.

Specific Reference:

- Young infants exhibit robust functional antibody responses and restrained IFN-γ production to SARS-CoV-2.;2021;Cell Rep Med.;Goenka A et al.

- Simultaneous evaluation of antibodies that inhibit SARS-CoV-2 variants via multiplex assay.;2021;JCI Insight.;Lopez E et al.

- SARS-CoV-2 requires cholesterol for viral entry and pathological syncytia formation.;2021;Elife.;Sanders DW et al.

- Structure and mechanism of SARS-CoV-2 Spike N679-V687 deletion variant elucidate cell-type specific evolution of viral fitness.;2021;bioRxiv;Gupta K et al.

- Within-host evolution of SARS-CoV-2 in an immunosuppressed COVID-19 patient: a source of immune escape variants.;2021;Viruses.;Weigang S et al.

Disclaimer:

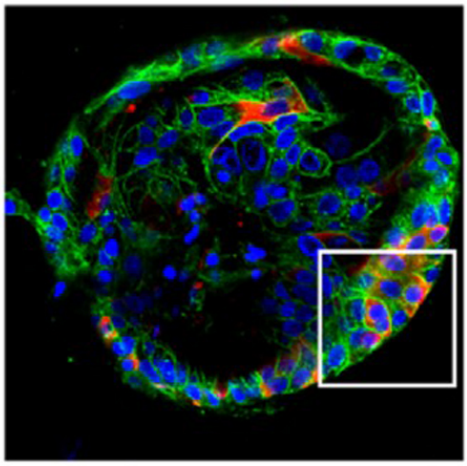
intellectual property that may address, or is necessary for, use of this product other than as expressly set forth above.

The terms of this limited use license and warranties shall be governed by the laws of the Commonwealth of Pennsylvania.

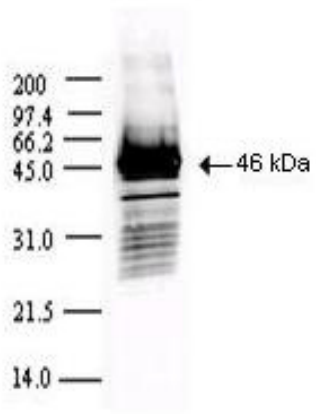
Related Products:

100-401-A52	Anti-SARS-CoV Nonstructural Protein 3 (nsp3) (RABBIT) Antibody - 100-401-A52
100-401-A53	Anti-SARS-CoV Nonstructural Protein 8 (nsp8) (RABBIT) Antibody - 100-401-A53
100-401-A54	Anti-SARS-CoV Nonstructural Protein 13 (nsp13) (RABBIT) Antibody - 100-401-A54
100-401-A55	Anti-SARS-CoV Membrane (M) Protein (RABBIT) Antibody - 100-401-A55
200-401-A51	Anti-SARS-CoV 3CL Protease (RABBIT) Antibody - 200-401-A51
611-1302	Anti-RABBIT IgG (H&L) (GOAT) Antibody Peroxidase Conjugated - 611-1302
618-103-012	Anti-FERRET IgG (gamma chain) (GOAT) Antibody Peroxidase Conjugated - 618-103-012
B304	NORMAL GOAT SERUM (NGS) - B304
MB-070	Blocking Buffer for Fluorescent Western Blotting - MB-070

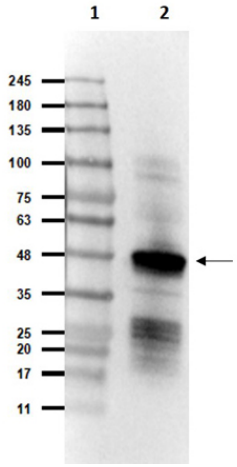
Data:



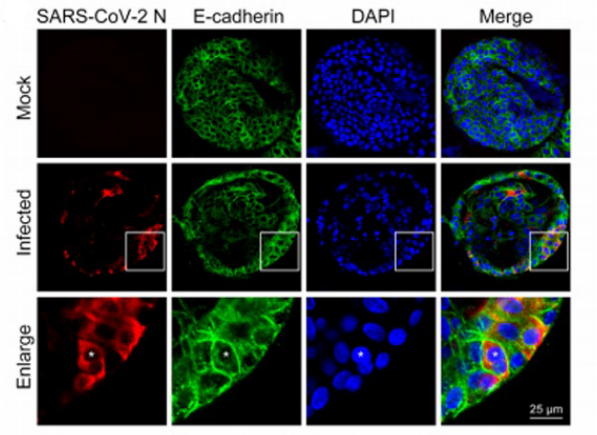
Immunofluorescence of Rabbit Anti-SARS-CoV Nucleocapsid (N) Antibody.
Tissue: human Liver ductal organoids.
Fixation: 4% PFA.
Permeabilization: 0.25% Triton X-100.
Antigen retrieval: not required.
Primary antibody: Rabbit Anti-SARS-CoV (N) Antibody and Mouse Anti-E-Cadhedrin Antibody at 1:500 overnight at 2-8°C.
Secondary antibody: Donkey Anti-Rabbit IgG CY3 Conjugated; Donkey Anti-Mouse IgG AlexaFluor 488 Conjugated for 1hr at RT.
Nuclear Counterstain: DAPI.
Staining of Infected and Merged: SARS-CoV Red signal, E-Cadhedrin green signal, with DAPI (blue) nuclear counterstain.
[Zhao et al. (2020)]



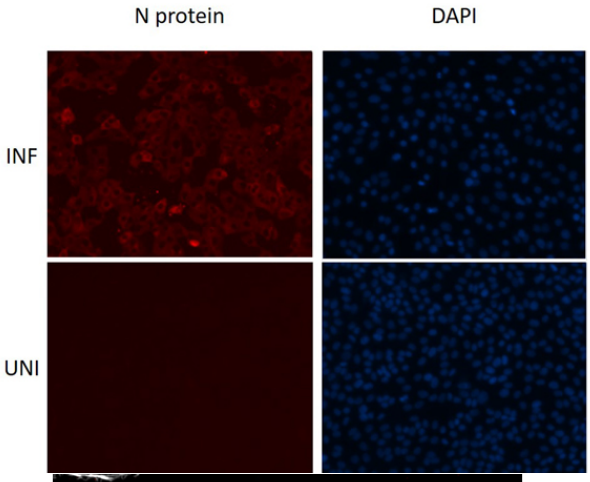
Western blot using Rockland's Protein A Purified anti-SARS CoV Nucleocapsid (N) protein antibody shows detection of a 46-kDa band corresponding to the protein. Approx. 100 ng of protein was loaded for SDS-PAGE and transferred onto nitrocellulose. The blot was incubated with a 1:5,000 dilution of the antibody at room temperature for 1 h followed by detection using IRDye™800 labeled Goat-a-Rabbit IgG [H&L] (611-132-122) diluted 1:10,000. The fluorescence image was captured using the Odyssey® Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.



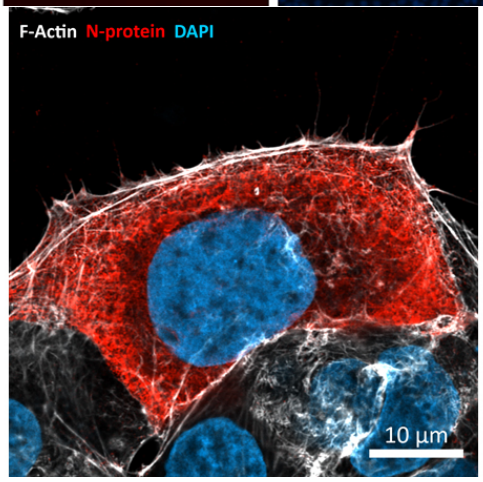
Western Blot of Rabbit Anti-SARS CoV Nucleocapsid (N) Protein Antibody.
Lane 1: Opal Prestained Molecular Weight Marker (p/n MB-210-0500).
Lane 2: SARS CoV Nucleocapsid (N) Protein [20ng].
Primary Antibody: Anti-SARS CoV Nucleocapsid (N) Protein Antibody at 1.0µg/mL overnight at 2-8°C.
Secondary Antibody: Goat Anti-Rabbit IgG HRP (p/n 611-1302) at 1:40000 for 30mins at RT.
Block: BlockOut Buffer (p/n MB-073).
Predicted MW: ~46kDa.
Observed MW: ~48kDa.



Immunofluorescence of Rabbit Anti-SARS-CoV Nucleocapsid (N) Antibody.
Tissue: human Liver ductal organoids.
Fixation: 4% PFA.
Permeabilization: 0.25% Triton X-100.
Antigen retrieval: not required.
Primary antibody: Rabbit Anti-SARS-CoV (N) Antibody and Mouse Anti-E-Cadhedrin Antibody at 1:500 overnight at 2-8°C.
Secondary antibody: Donkey Anti-Rabbit IgG CY3 Conjugated; Donkey Anti-Mouse IgG AlexaFluor 488 Conjugated for 1hr at RT.
Nuclear Counterstain: DAPI.
Staining showing Mock and Infected tissue: SARS-CoV Red signal, E-Cadhedrin green signal, with DAPI (blue) nuclear counterstain. [Zhao et al. (2020)]



Immunofluorescence assay using Rabbit Anti-SARS-CoV Nucleocapsid (N) Antibody, showing viral protein detection. Vero E6 cells were either infected with the SARS-CoV-2 Washington isolate (INF) at an MOI of 0.1 or uninfected (UNI) for 24 hours. The cells were then fixed in 4% PFA and stained overnight at 4ºC with primary antibodies directed against SARS-CoV Nucleocapsid (N) at 1:1000 dilution. Imaged using an anti-rabbit secondary conjugated to AlexaFluor 568 [red] and Nuclear Counterstain DAPI [blue]. Image Courtesy of Mohsan Saeed Lab/Da-Yuan Chen, National Emerging Infectious Diseases Laboratories (NEIDL), Boston University.



Immunofluorescence of Rabbit Anti-SARS CoV (M) Protein Antibody.
Cells: Caco-2 cells 24 hours post-infection.
Fixation: 4% PFA.
Permeabilization: 0.3% Triton X-100.
Blocking: 5% fetal calf serum/PBS.
Primary Antibody: Anti-SARS-CoV nucleocapsid (N) protein at 1:1000 for 1 hour at RT.
Staining: N-Protein [Red], F-actin [White], DAPI, [Blue].
Imaged: Zeiss LSM800 microscope.
[Images Courtesy of AG Robert Grosse, Institute of Experimental and Clinical Pharmacology I, University of Freiburg/Svenja Ulferts and AG Georg Kochs Labs/Sebastian Weigang]. [Bouhaddou M et al. 2020]

Relevant Links:

GeneID - 1489678	http://www.ncbi.nlm.nih.gov/gene/1489678
NCBI - 30173007	http://www.ncbi.nlm.nih.gov/protein/30173007
UniProtKB - P59595	http://www.uniprot.org/uniprot/P59595