Datasheet for 200-401-A50

Sars Nucleocapsid Protein Antibody

Overview

Description:	Anti-SARS-CoV Nucleocapsid (N) Protein (RABBIT) Antibody - 200-401-A50
Item No.:	200-401-A50
Size:	500 µg
Applications:	ELISA, WB, FC, FISH, IF, IHC, LFA, Multiplex, Other
Reactivity:	Virus
Host Species:	Rabbit

Product Details

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.
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
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Reactivity:	Virus
Immunogen Type:	Recombinant Protein

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.
Purity/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. Detects the Nucleocapsid (N), Omicron, and BA.2 sub-variant. BLAST analysis was used to suggest reactivity with related Coronavirus proteins. Cross reactivity with homologues from other sources has not been determined.
Relevant Links:	• 200-401-A50 SDS • UniProtKB - P59595 • NCBI - 30173007 • GeneID - 1489678

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	FC, FISH, IF, IHC, LFA, Multiplex, Other (Based on references)
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.
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
FC:	User Optimized
IF:	User Optimized

IHC:	1:100-1:6000
WB:	1:2,000 - 1:10,000

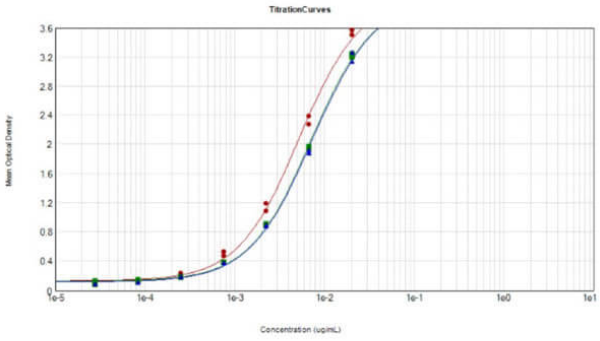
Formulation

Physical State:	Lyophilized
Concentration:	5.3 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None
Reconstitution Volume:	100 µL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

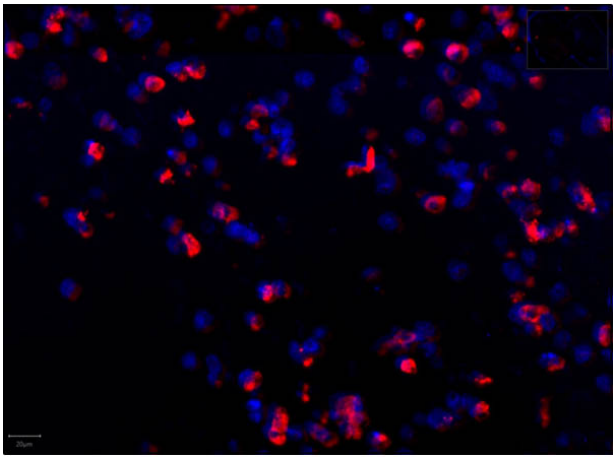
Shipping & Handling

Shipping Condition:	Ambient
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.
Expiration:	Expiration date is one (1) year from date of receipt.

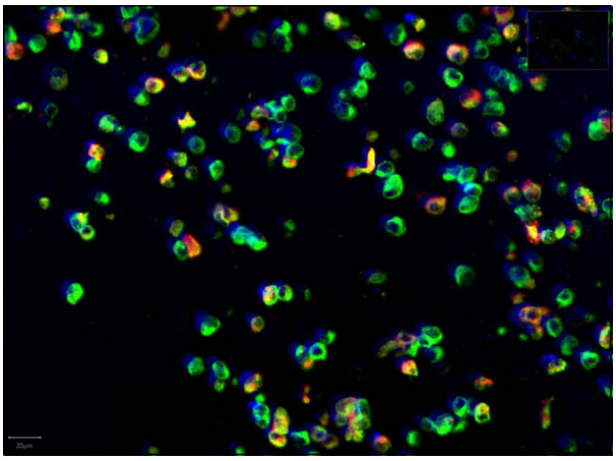
Images



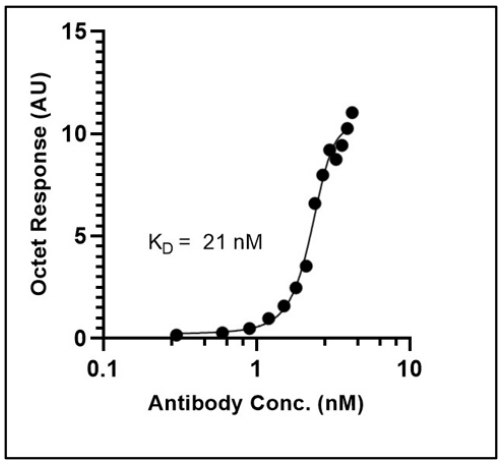
ELISA
ELISA results of Rabbit Anti-SARS-CoV Nucleocapsid (N) Protein Variants. Each well was coated in duplicate with 0.5µg/mL of SARS CoV-2 Canonical Nc [Red Line], SARS CoV-2 Omicron Nc [Green Line], SARS CoV-2 Omicron BA.2 Nc [Blue Line]. The starting dilution of antibody was 2µg/mL and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. SARS CoV-2 Nc IC50: 5 ng/mL, SARS CoV-2 Omicron Nc IC50: 7 ng/mL, SARS CoV-2 BA.2 Nc IC50: 7 ng/mL. Assay performed using Goat Anti-Rabbit IgG HRP conjugated (p/n 611-1302) and TMB substrate (p/n TMBE-1000).



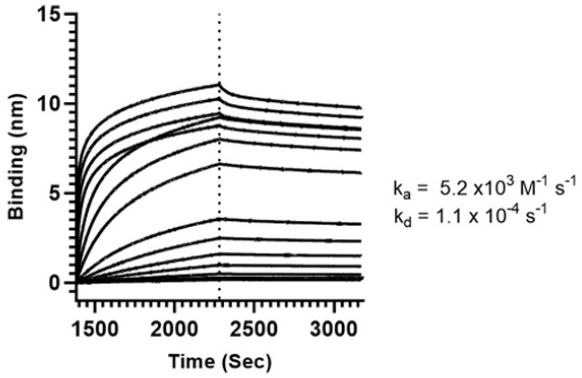
Immunofluorescence Microscopy
Immunofluorescence of SARS-CoV-2 infection in FFPE cell pellets from in vitro cultured human lung cells infected with SARS-CoV-2. Anti-SARS-CoV-2 Nucleocapsid Protein antibody (p/n 200-401-A50) is visualized with PhenoCycler reporters in red and DAPI-stained chromatin in blue. Image provided by Akoya Biosciences.



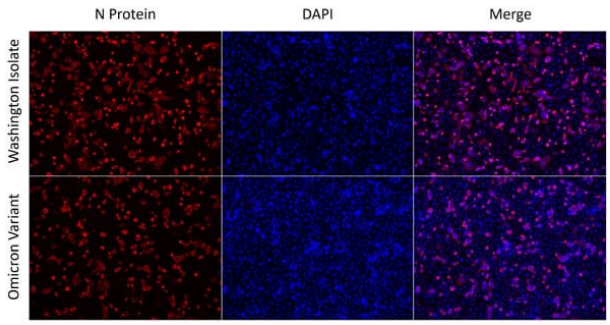
Immunofluorescence Microscopy
Immunofluorescence of SARS-CoV-2 infection in FFPE cell pellets from in vitro cultured human lung cells infected with SARS-CoV-2. Costaining with Anti-SARS-CoV-2 Nucleocapsid Protein antibody (p/n 200-401-A50) in red, Anti-SARS-CoV-2 Spike S1 antibody RBD antibody (antibodies-online p/n ABIN6952546) in green, and DAPI-stained chromatin in blue. Image provided by Akoya Biosciences.



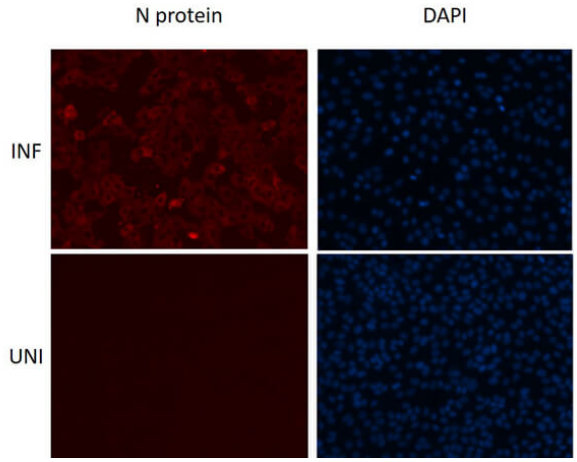
ELISA
Fitting of Kinetic Data to bivalent binding site model. Steady state signals are fitted to a bivalent binding site model. A 4-parameter non-linear regression curve was used for the integration of the data. The Rabbit Anti-SARS-CoV Nucleocapsid (N) Protein Antibody (p/n 200-401-A50) presents a KD=21nM (affinity constant) to SARS-CoV Nucleocapsid (N) Protein.



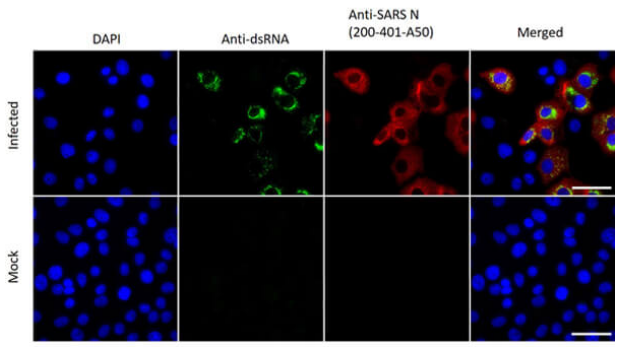
ELISA
Binding mode and kinetic analysis of Rabbit Anti-SARS-CoV Nucleocapsid (N) Protein Antibody (p/n 200-401-A50). Using Octet R4, SARS COV-1N was immobilized at 20 µg/mL on an AR2G biosensor and exposed to Rabbit Anti-SARS-CoV Nucleocapsid (N) Protein Antibody over a range of 2.0 nM – 16.7 µM. Each curve represents the acquired signal of its protein concentration. The interaction fits to a bivalent binding site kinetic model. The association constant (ka=5.2x10^3 M^-1 s^-1) and dissociation constant (kd=1.1x10^-4 s^-1) values are depicted in the graph. The assay was performed as per the manufacturer's protocol.



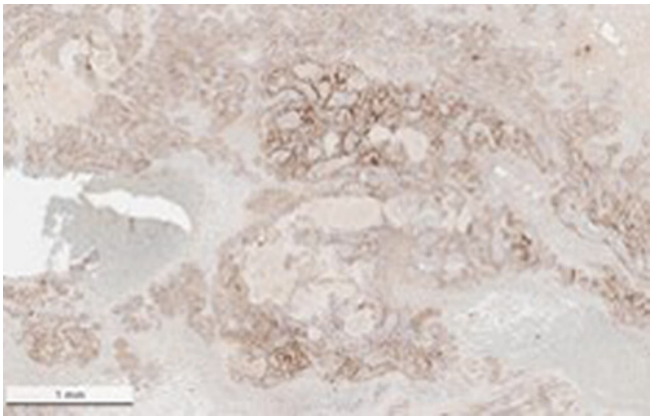
Immunofluorescence Microscopy
Immunofluorescence assay using Rabbit Anti-SARS-CoV Nucleocapsid (N) Antibody, showing viral protein detection. A549 cells over-expressing ACE2 were either infected with the SARS-CoV-2 Washington isolate or Omicron Variant at an MOI of 0.5 for 24 hours. The cells were then fixed in 10% Formalin and stained overnight at 4ºC with primary antibodies directed against SARS-CoV Nucleocapsid (N) (Rockland p/n 200-401-A50) 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.



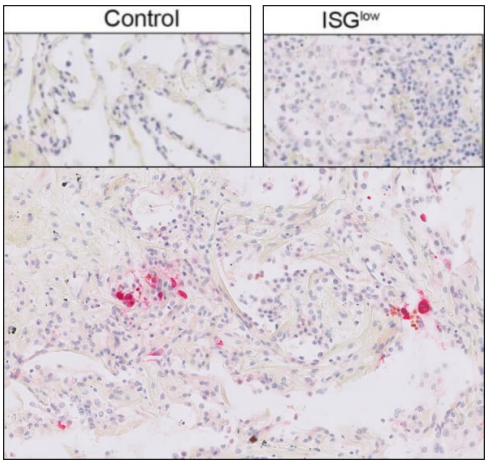
Immunofluorescence Microscopy
Immunofluorescence assay using Rabbit Anti-SARS-CoV Nucleocapsid (N) Antibody, showing viral protein detection. Vero E6 cells were either infected with the SARS-CoV-2 Washington isolate (INF) at an MOI of 0.1 or uninfected (UNI) for 24 hours. The cells were then fixed in 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.



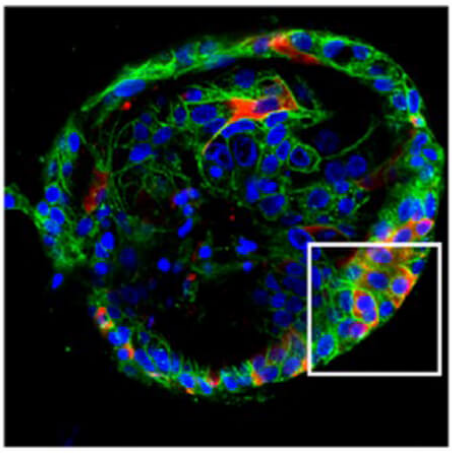
Immunofluorescence Microscopy
Immunofluorescence assay using Rabbit Anti-SARS-CoV Nucleocapsid (N) Antibody, showing viral protein synthesis. VeroE6 cells were infected with rSARS-CoV-2. Mock: non-infected cells. At 48 h.p.i, cells were fixed and prepared for immunofluorescence staining with primary antibodies directed against double-stranded RNA (dsRNA) [green] and SARS-CoV Nucleocapsid (N) [red]. Nuclear Counterstain DAPI [blue].



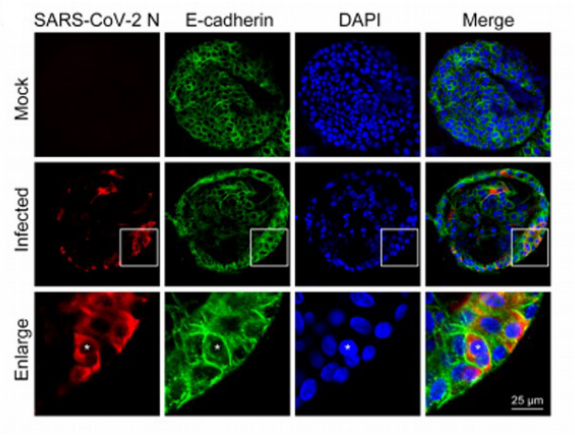
Immunohistochemistry
Immunohistochemistry results showing positive staining in placenta tissue using Anti-SARS-CoV Nucleocapsid (N) Antibody.



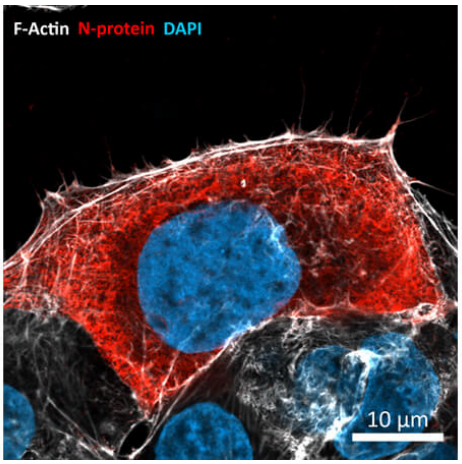
Immunohistochemistry
Immunohistochemistry of Rabbit Anti-SARS CoV Nucleocapsid Antibody. Pre-treated tissue: (A) human lung control, (B) interferon stimulated genes (ISG) low, (C) ISG high human COVID-19 lung: interferon stimulated genes (ISGs). [One pattern shows high expression of interferon stimulated genes (ISGs) and cytokines, high viral loads and limited pulmonary damage, the other pattern showes severely damaged lungs, low ISGs, low viral loads and abundant immune infiltrates.] Primary Antibody: Anti-SARS-CoV at 1:6400. Staining and Detection with Bond Polymer Refine Red Detection. [Courtesy of Mertz K. Profiles of COVID-19 lungs.]



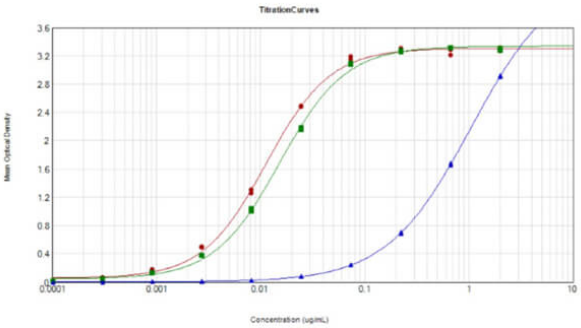
Immunofluorescence Microscopy
Immunofluorescence of Rabbit Anti-SARS-CoV Nucleocapsid (N) Antibody. Tissue: human Liver ductal organoids. Fixation: 4% PFA. Permeabilization: 0.25% Triton X-100. Antigen retrieval: not required. Primary antibody: Rabbit Anti-SARS-CoV (N) Antibody and Mouse Anti-E-Cadhedrin 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)]



Immunofluorescence Microscopy
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)]

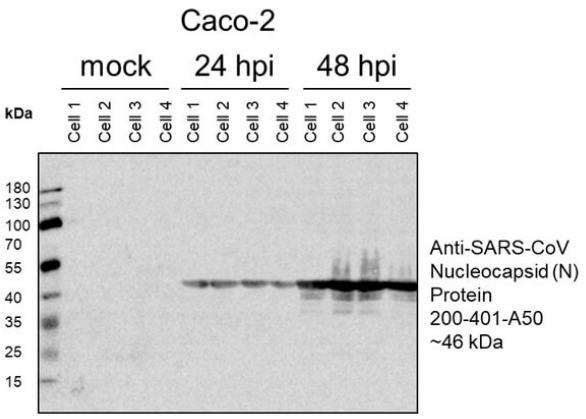


Immunofluorescence Microscopy
Immunofluorescence of Rabbit Anti-SARS CoV (M) Protein Antibody. Cells: Caco-2 cells 24 hours post-infection. Fixation: 4% PFA. Permeabilization: 0.3% Triton X-100. Blocking: 5% fetal calf serum/PBS. Primary Antibody: Anti-SARS-CoV nucleocapsid (N) protein at 1:1000 for 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]



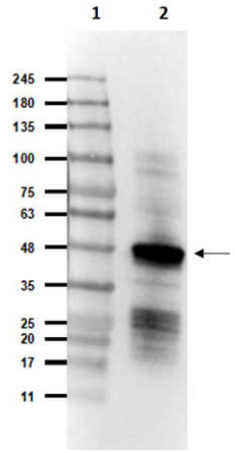
ELISA

ELISA results of Rabbit Anti-SARS-CoV Nucleocapsid (N) Protein. Each well was coated in duplicate with 0.5µg/mL of SARS CoV-1 [Red Line], SARS CoV-2 Nc [Green Line], MERS CoV N [Blue Line]. The starting dilution of antibody was 2µg/ml and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. SARS CoV-1N IC50: 11ng/mL, SARS CoV-2N IC50: 15ng/mL. Assay performed using Goat Anti-Rabbit IgG HRP conjugated (p/n 611-1302) and TMB substrate (p/n TMBE-1000).



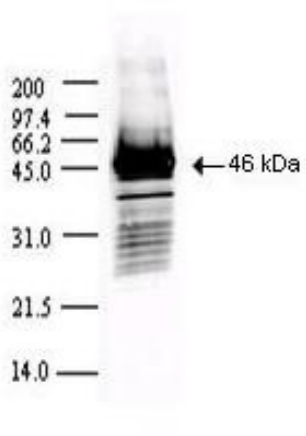
Western Blot

Western Blot of Rabbit Anti-SARS-CoV (N) Antibody tested on Caco-2 Cell Lysates before (mock), 24, and 48 hours post infection (hpi). Primary Antibody: Anti-SARS-CoV Nucleocapsid (N) Antibody. Observed MW: ~46kDa in infected cells. [Courtesy of Sebastian Weigang - Kochs Labs]



Western Blot

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.



Western Blot
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.

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