

**Datasheet for 600-401-A50****Sars Nucleocapsid Protein Antibody****Overview**

<b>Description:</b>	Anti-SARS-CoV Nucleocapsid (N) Protein (RABBIT) Antibody - 600-401-A50
<b>Item No.:</b>	600-401-A50
<b>Size:</b>	100 µg
<b>Applications:</b>	ELISA, IF, Other
<b>Reactivity:</b>	SARS-CoV
<b>Host Species:</b>	Rabbit

**Product Details**

<b>Background:</b>	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.
<b>Synonyms:</b>	rabbit anti-Sars Nucleocapsid Protein Antibody, rabbit anti-Sars-CoV Nucleocapsid (N) Protein Antibody, N antibody, N structural protein antibody, COVID19 antibody, Nucleocapsid protein antibody, Nucleoprotein antibody, SARS coronavirus N protein antibody, SARS CoV-2 antibody, SARSCoV2 antibody, Severe acute respiratory syndrome antibody, A50 Antibody, A50 SARS, COVID
<b>Host Species:</b>	Rabbit
<b>Clonality:</b>	Polyclonal
<b>Format:</b>	IgG

**Target Details**

<b>Reactivity:</b>	SARS-CoV
<b>Immunogen Type:</b>	Recombinant Protein
<b>Immunogen:</b>	This affinity 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.

**Purity/Specificity:** Anti-SARS-CoV-1 (N) affinity purified antibody is directed against SARS Coronavirus Nucleocapsid (N) protein. The product was purified from monospecific antiserum by immunoaffinity chromatography over SARS CoV-2 (N) resin. 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:**

- [UniProtKB - P59595](#)
- [NCBI - 30173007](#)
- [GeneID - 1489678](#)

## Application Details

**Tested Applications:** ELISA, IF

**Suggested Applications:** Other (Based on references)

**Application Note:** Anti-SARS-CoV-1 purified antibody has been tested for use in ELISA, immunofluorescence, and western blot. 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. This antibody can be useful in ELISA and lateral flow format to detect virus in extracts from nasal and throat swabs and saliva. IF can be useful to determine the presence or absence of virus entering cells especially when anti-viral drugs are applied. IHC studies can be performed on biopsies, detecting the coronavirus in lung, liver, bile duct, and placenta tissue. And may have the ability to neutralize the virus and thereby protect cells from the uptake of live virus. This antibody may be useful 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:** User Optimized

**WB:** 1:500 - 1:2,000

## Formulation

**Physical State:** Liquid (sterile filtered)

**Concentration:** 1.2 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

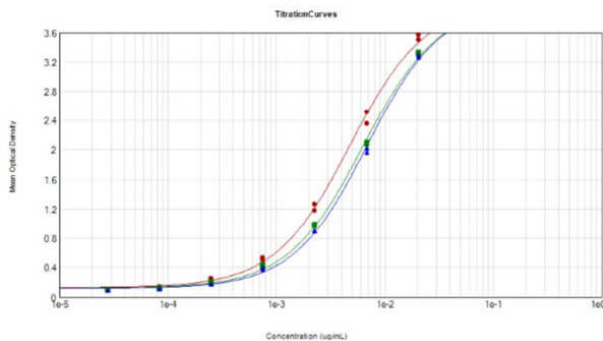
## Shipping & Handling

**Shipping Condition:** Dry Ice

**Storage Condition:** Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. 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.

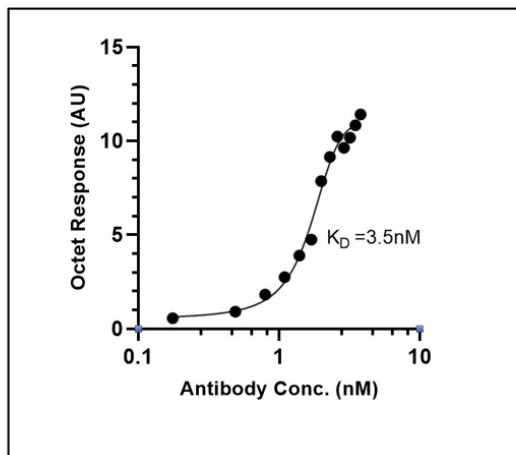
**Expiration:** Expiration date is one (1) year from date of receipt.

## Images



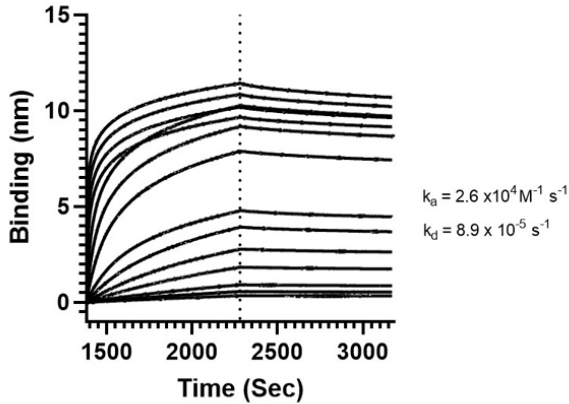
### ELISA

ELISA results of Rabbit Anti-SARS-CoV Nucleocapsid (N) Protein Variants. Each well was coated in duplicate with 0.5 µg/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 0.55 µg/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: 6 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).



### 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 600-401-A50) presents a  $K_D = 3.5nM$  (affinity constant) to SARS-CoV Nucleocapsid (N) Protein.

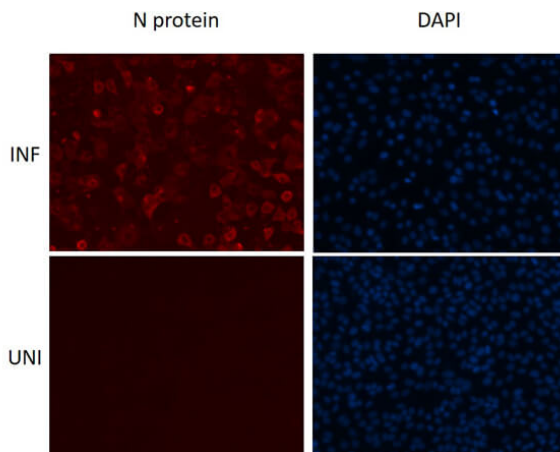
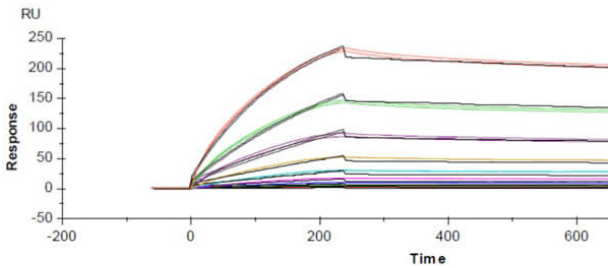


### ELISA

Binding mode and kinetic analysis of Rabbit Anti-SARS-CoV Nucleocapsid (N) Protein Antibody (p/n 600-401-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 0.8 nM – 6.4 µM. Each curve represents the acquired signal of its protein concentration. The interaction fits to a bivalent binding site kinetic model. The association constant ( $k_a=2.6 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ ) and dissociation constant ( $k_d=8.9 \times 10^{-5} \text{ s}^{-1}$ ) values are depicted in the graph. The assay was performed as per the manufacturer’s protocol.

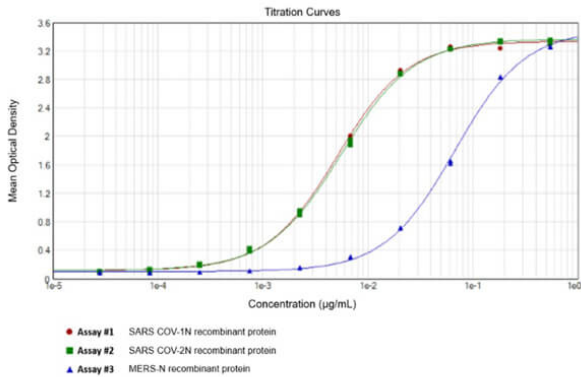
### Surface Plasmon Resonance (SPR)

Surface Plasmon Resonance (SPR) Data of Rabbit Anti-SARS-CoV Nucleocapsid (N) Antibody. SARS-CoV-2 Nucleocapsid protein was immobilized on a carboxymethyl dextran sensor chip. Ten (10) dilutions of Anti-SARS-CoV Nucleocapsid (N) Antibody (colors) were flowed across the chip at 30 µL/min with 240 s contact time and then 720 s dissociation time. Fitted line (black) constants are:  $k_{on} = 2.5 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$  and  $k_{off} = 1.6 \times 10^{-4} \text{ s}^{-1}$ . The Equilibrium Dissociation constant (KD) of the antibody on several assays was 6.5 nM. (Average primary antibody KD = 1 µM).



### 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 4°C 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.



### ELISA

ELISA results of Rabbit Anti-SARS-CoV Nucleocapsid (N) Protein Antibody. Each well was coated in duplicate with 50ng of SARS CoV-1 N [Red Line], SARS CoV-2 N [Green Line], MERS N [Blue Line]. The starting dilution of antibody was 0.55µg/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-1 N IC50: 5ng/mL, SARS CoV-2 N IC50: 6ng/mL, MERS N IC50: 67ng/mL. Assay performed using Goat Anti-Rabbit IgG HRP conjugated (p/n 611-1302) and TMB substrate (p/n TMBE-1000).

## References

- Zhao B. et al. Recapitulation of SARS-CoV-2 Infection and Cholangiocyte Damage with Human Liver Organoids. *Protein Cell* (2020)
- Burbelo P.D. et al. Detection of Nucleocapsid Antibody to SARS-CoV-2 is More Sensitive than Antibody to Spike Protein in COVID-19 Patients. *medrxiv* (2020)
- Liu Y. et al. Functional and Genetic Analysis of Viral Receptor ACE2 Orthologs Reveals Broad Potential Host Range of SARS-CoV-2. *Proc Natl Acad Sci U S A* (2020)
- V'kovski P. et al. Disparate temperature-dependent virus – host dynamics for SARS-CoV-2 and SARS-CoV in the human respiratory epithelium. *PLoS Biol.* (2020)
- Grant BD, Anderson CE, Williford JR, et al. A SARS-CoV-2 Coronavirus Nucleocapsid Antigen-Detecting Half-Strip Lateral Flow Assay Towards the Development of Point of Care Tests Using Commercially Available Reagents. *Anal Chem.* (2020)

## Disclaimer

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