



Product Specification Sheet

SARS-COV-2/COVID-19 Nucleocapsid antibody

Cat # NCOVP11-A	Rabbit Anti-COVID-19 Nucleocapsid protein antibody	SIZE: 100 µg
Cat # NCOVP21-A	Rabbit Anti-COVID-19 Nucleocapsid protein antibody	SIZE: 100 µg

SARS-CoV-2 virus (SARS-CoV-2), is a novel coronavirus emerged as a human respiratory pathogen and causing the 2020 pandemic named COVID-19. The SARS-CoV-2 genome is closely related to 2 bat-derived severe acute respiratory syndrome (SARS)-like coronaviruses (88% identity) and more distantly from 2 other human pathogenic coronaviruses, SARS-CoV (~79% identity) and MERS-CoV (~50% identity).

The genome of the coronavirus encodes 23 putative proteins including 4 major structural proteins: nucleocapsid [N protein], spike [S protein], membrane [M] and small envelope proteins [E].

The S protein is a glycoprotein essential for viral attachment to the host cell surface receptors and translocation into the infected cells; trimers of the S protein make up the spikes of the virus. The S protein is cleaved in host cells into S1 and S2 subunits; S1 protein binds the host receptor, while S2 mediates membrane fusion. A minimal receptor-binding domain [RBD] located in the S1 protein (aa. 318-510) can combine with the ACE2 receptor on host epithelial cells. While the S1 subunit of SARS-CoV-2 shares around 70% identity to that of the 2 bat SARS-like CoVs and human SARS-CoV, the core domains of RBD (excluding the external subdomain) are highly conserved.

SARS Nucleocapsid has been shown to be highly immunogenic and abundantly produced, therefore is used as a possible target for immunodiagnosics.

Source of Antigen and Antibodies

Uniprot: P0DTC9

Host: Rabbit

Clonality: Polyclonal

Immunogen:

NCOVP11-A: Synthetic peptide within amino acids (200-300) of COVID-19 NP

NCOVP21-A: Synthetic peptide within amino acids (350-419) of COVID-19 NP

Purification: Ammonium sulfate followed by antigen affinity chromatography

Cross reactivity:

NCOVP11-A: The peptide sequence has 100% homology with Bat Coronavirus and SARS Coronavirus NP, no significant homology with MERS or common cold strains 229E, NL63, OC43, or HKU1

NCOVP21-A: Peptide sequence has 87% homology with Bat Coronavirus and SARS Coronavirus NP, no significant homology with MERS or common cold strains 229E, NL63, OC43, or HKU1

Form & Storage of Antibodies

Affinity pure IgG Solution

Concentration: 1.0 mg/ml Volume: 100 µl

Supplied in PBS, pH 7.4

The antibody can be made available conjugated to HRP, Biotin, FITC, or colloidal gold on request

Lyophilized powder

Lyophilized from a formulation containing PBS, pH 7.4 +3% Trehalose. Reconstitute in 100 µl distilled water to 1 mg/ml

Storage:

Short-term: 4°C for 3 months

Long-term: at -20°C or below in suitable aliquots after reconstitution for 1 year. Do not expose to multiple freeze/thaw cycles or store working, diluted solutions. Glycerol may be added to a final concentration of 50% and antibodies can be stored un-aliquoted at -20°C.

Recommended Usage

Pairing information: **NCOVP11-A** and **NCOVP21-A** did not demonstrate the ability to pair in ELISA. **NCOVP11-A** has a higher affinity and is recommended for applications which require one antibody or testing pairing with other antibodies.

ELISA: Assay dependent concentration. Typically ranges from 0.1-2.0 µg/ml as a capture or detection antibody. *NCOVP11-A is used as a capture antibody at 1 µg/ml in a Sandwich ELISA (ADI cat#RV-405500) with a sensitivity of ~20 pg/ml.

Lateral Flow: Assay dependent use as a coating antibody. Minimum recommended concentration of 5 µg/ml when conjugated to colloidal gold in a salt free buffer at pH 7-9.

Western blot: 0.5-2 µg/ml. Expected band size: 48-55 kDa.

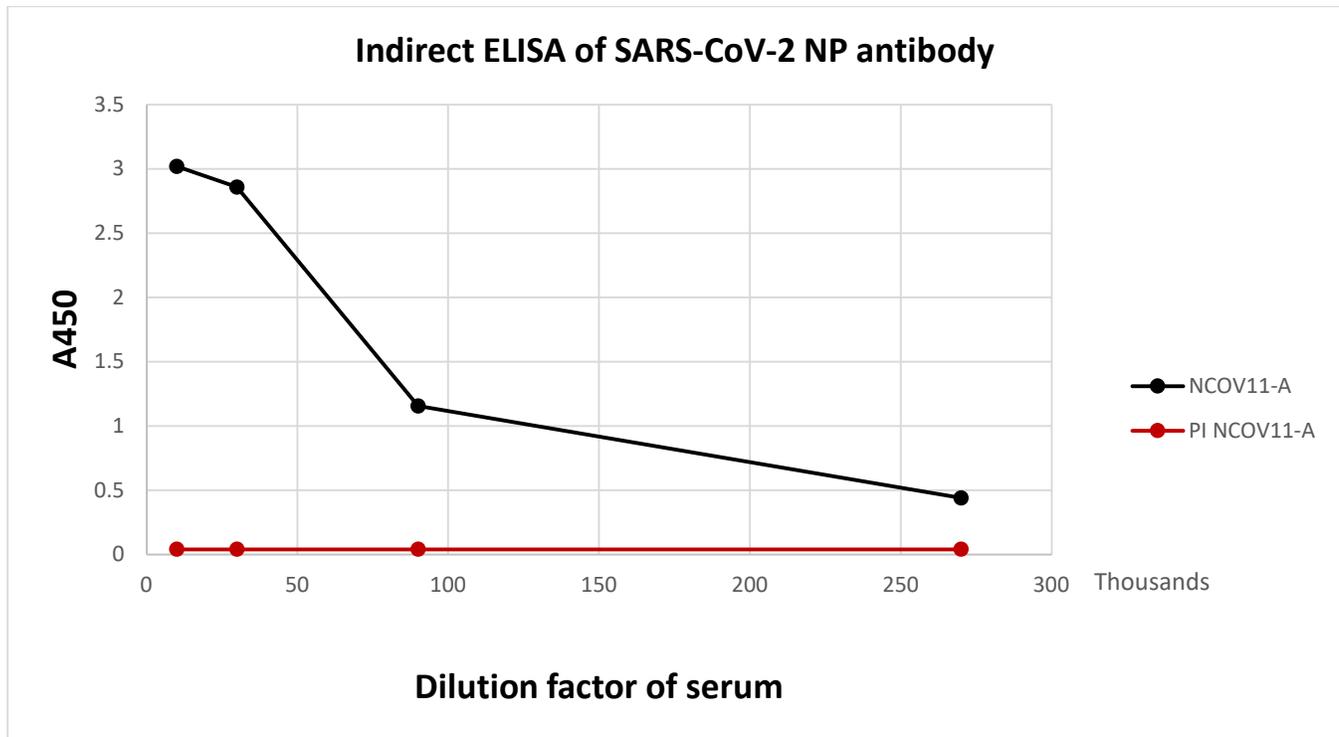
IHC/ICC: 1-10 µg/ml

****This product is for In vitro research use only.***

Related materials available from ADI

Catalog#	Description
NCOV15-R-1	Recombinant COVID-19 Nucleocapsid
NCOVS-1	Synthetic COVID-19 antigen (For Lateral Flow & ELISA serology assays)
RV-405000	SARS-COV-2 Neutralizing antibody/Inhibitor Compound screening ELISA Kit
RV-404120	Recombinant Mouse anti COVID-19/2019-nCoV Nucleocapsid IgG ELISA Kit
RV-404150	Recombinant Monkey anti COVID-19/2019-nCoV Nucleocapsid IgG ELISA Kit
RV-404250	Recombinant Monkey anti COVID-19/2019-nCoV Spike protein 1(S1) IgG ELISA Kit
RV-405200	Recombinant Human Anti SARS-CoV-2 (COVID-19) Spike protein 1(S1) IgG ELISA Kit
NCOVP11-A	050820IA

ELISA validation of SARS-CoV-2 Nucleocapsid antibody

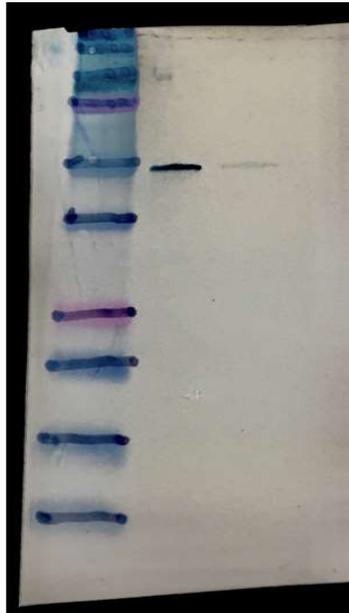


ELISA: Full length SF9 expressed SARS-CoV-2 Nucleocapsid was coated to ELISA microwell plates. Rabbit serum was diluted and incubated for 1 hour. The microwells were then washed and Goat anti-Rabbit IgG Fc-HRP was then added and incubated for 30 minutes. The microwells were washed and TMB was added and incubated for 15 minutes, the reaction was stopped with Sulfuric acid and read at 450 nm.

Serum dilution	NP ab (A ₄₅₀)	Pre-Immune serum (A ₄₅₀)
10,000	>3.02	0.04
30,000	2.857	0.04
90,000	1.156	0.04
270,000	0.44	0.04

Results indicate that the linear peptide immunogen used for antibody production can bind to a full length, soluble, glycosylated, protein in native confirmation at high titers.

Western Blot validation of SARS-CoV-2 Nucleocapsid antibody



Western Blot: Full length *E.coli* expressed SARS-CoV-2 Nucleocapsid was loaded at total protein concentrations of 5 ng, 0.5 ng, and 0.05 ng into an 'Any Kd' (Biorad) SDS-PAGE gel. The gel was run for approximately 1 hour at 200V and transferred to a 0.45 μ m nitrocellulose membrane using the 'Mixed MW' settings on a Transblot Turbo (Biorad). The membrane was blocked for 1 hour with 5% BSA. **NCOVP11-A** was diluted to 1 μ g/ml in TBST +0.1% BSA and incubated for 1 hour. After 1 hour, the membrane was washed and incubated with Goat anti-Rabbit IgG HRP for 1 hour. The membrane was washed and insoluble TMB colorimetric substrate was added and incubated. The antibody detected a single band of ~48 kDa and demonstrates a visual sensitivity of less than 0.5 ng of total protein.