ANTI-NUCLEOCAPSID (N) RABBIT MONOCLONAL AB #VYN7

CATALOG & PRICING

Cat. #	Conjugation	Size
VYN7-20	None	20 µL
VYN7-50		50 µL
VYN7-100		100 µL
VYN7B-20	Biotin	20 µL
VYN7B-50		50 µL
VYN7B-100		100 µL
VYN7H-20	Horseradish Peroxidase	20 µL
VYN7H-50		50 µL
VYN7H-100		100 µL

ANTIGEN BACKGROUND

The single-stranded, plus-sense severe acute respiratory syndrome coronavirus 2 (SARS–CoV-2) genome encodes nonstructural replicase polyproteins as well as structural proteins such as spike (S), nucleocapsid (N), membrane (M) and envelop (E) proteins (Zhou et al., 2020). The N protein is abundantly expressed and highly immunogenic during SARS-CoV-2 infection (Cong et al., 2020). The middle or C-terminal region of the N protein has been shown to elicit antibody production during the immune response (Dutta et al., 2020). Although the surface electrostatic potential characteristics of SARS-CoV-2 N are different from other coronavirus N proteins (Kang et al., 2020), the sequences are conserved among these proteins (Dutta et al., 2020). Besides S protein, N protein is also considered a leading target antigen for vaccine development (Chen et al., 2020; Dutta et al., 2020).

Chen, W.H., Strych, U., Hotez, P.J., and Bottazzi, M.E. (2020). The SARS-CoV-2 Vaccine Pipeline: An Overview. Curr Trop Med Rep, 1-4.

Cong, Y., Ulasli, M., Schepers, H., Mauthe, M., V'Kovski, P., Kriegenburg, F., Thiel, V., de Haan, C.A.M., and Reggiori, F. (2020). Nucleocapsid Protein Recruitment to Replication-Transcription Complexes Plays a Crucial Role in Coronaviral Life Cycle. J Virol 94.

Dutta, N.K., Mazumdar, K., and Gordy, J.T. (2020). The Nucleocapsid Protein of SARS-CoV-2: A Target for Vaccine Development. J Virol 94.

Kang, S., Yang, M., Hong, Z., Zhang, L., Huang, Z., Chen, X., He, S., Zhou, Z., Zhou, Z., Chen, Q., et al. (2020). Crystal structure of SARS-CoV-2 nucleocapsid protein RNA binding domain reveals potential unique drug targeting sites. Acta Pharm Sin B 10, 1228-1238.

Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang, C.L., et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270-273.

PRODUCT FEATURES

- Recombinant antibodies with minimum batch-to-batch variations
- Thoroughly tested using SARS-CoV-2, human nasopharyngeal swabs, and rN
- No cross-reactivity with SARS or MERS

PRODUCT DETAILS

ALIASES OF THE PROTEIN (ANTIGEN)
Severe acute respiratory syndrome coronavirus 2 nucleocapsid; 2019 novel coronavirus nucleoprotein; SARS-CoV-2 NP; SARS-CoV-2 N protein

ANTIGEN BACKGROUND
UniProt Entry: P59595

APPLICATION INFORMATION
Antigen Molecular Weight: 46 kDa
Clonality: Rabbit monoclonal antibody
Clone ID: V700-G5
Species Reactivity: SARS-CoV-2
Applications Tested: Western blotting (WB), ELISA, Immunoprecipitation (IP)

ANTIGEN SUBCELLULAR LOCATION
Virion; Host ER-Golgi intermediate compartment; host Golgi apparatus; host perinuclear region

IMMUNOGEN
Recombinant SARS-CoV-2 nucleocapsid protein

ISOTYPE
Rabbit IgG

SHIPPING
Supplied in PBS (pH 7.5) containing 0.01% sodium azide

STORAGE
Store at – 20°C

RECOMMENDED DILUTIONS
Western blotting (WB): 1:2,000
ELISA: 1:10,000
Immunoprecipitation (IP): 1:50-1:100

VALIDATION DATA

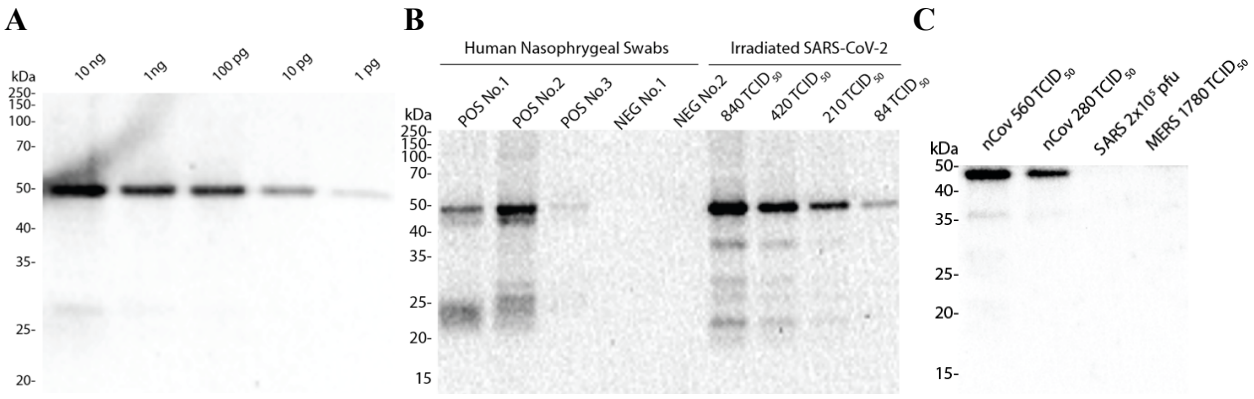

Figure 1. Antibody specificity and cross-reactivity test. Serial dilutions of recombinant SARS-CoV-2 N protein (Cat# VPN2) (**A**), 3 positive and 2 negative human nasopharyngeal swab specimens (20 µL) (**B**), and different concentrations of gamma-irradiated SARS-CoV-2 (nCoV, **B&C**), SARS-CoV (SARS) (**C**), and MERS (**C**) were lysed in Viral Lysis Buffer (Cat# VL101) and blotted with rabbit monoclonal anti-N primary antibody (Cat# VY7N, 1:2,000). HRP-conjugated highly cross-adsorbed goat anti-rabbit secondary antibody (Cat# 202, 1:2,000) was applied. The blots were incubated using the PiQ™ ECL Substrate Kit (Cat# 636) and imaged using a chemiluminescence digital imager. Note that 1) the NP POS No.3 swab had a very low viral load; and 2) There was no cross-reactivity of the antibody with SARS or MERS.

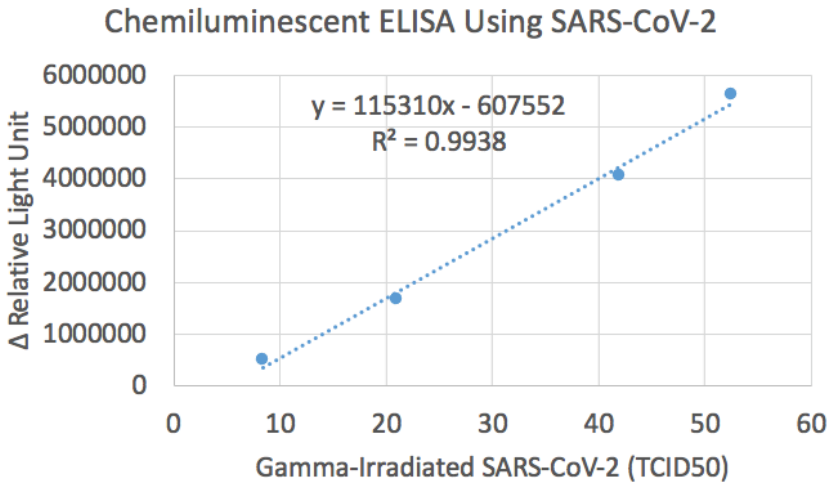

Figure 2. Chemiluminescent ELISA using SARS-CoV-2. Different concentrations of gamma-irradiated SARS-CoV-2 viruses were lysed in the Viral Lysis Buffer (Cat# VL101) and coated (150 µL) on microplate wells. Rabbit monoclonal anti-N antibody (Cat# VYN7, 1:10,000 dilution) and HRP-conjugated highly cross-adsorbed goat anti-rabbit secondary antibody (Cat# 202, 1:20,000) were used to detect the SARS-CoV-2 N protein. PiQ™ ECL substrates (Cat# 636) were used to produce chemiluminescent signals. RLU, relative light unit. ΔRLU = RLU of the well with the primary antibody - RLU of the corresponding well without the primary antibody, everything else being equal.

NEG(Δ RLU)	POS(Δ RLU)	LOGNEG	LOGPOS
2305	8617798	3.362671	6.935396
2101	1645513	3.322426	6.216301
269	97840	2.429752	4.990516
1337	53935	3.126131	4.731871
708	280303	2.850033	5.447628
2878	109956	3.459091	5.041219

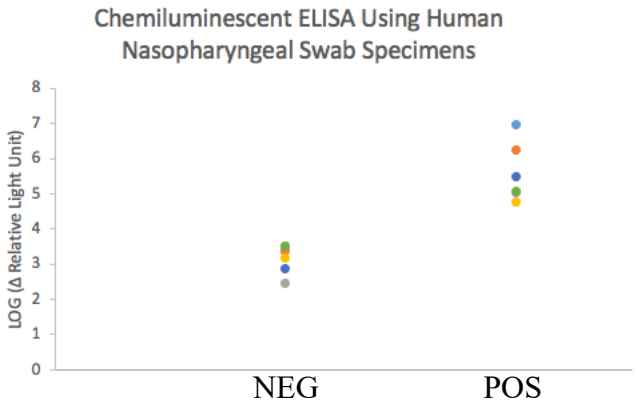

Figure 3. Chemiluminescent ELISA using human nasopharyngeal swab specimens. Six negative (NEG) and 6 positive (POS) human nasopharyngeal swab specimens were lysed in the Viral Lysis Buffer (Cat# VL101) and coated on microplate wells. Rabbit monoclonal anti-N antibody (Cat# VYN7, 1:10,000 dilution) and HRP-conjugated highly cross-adsorbed goat anti-rabbit IgG secondary antibody (Cat# 202, 1:20,000) were used to detect SARS-CoV-2 N protein. RLU, relative light unit. ΔRLU = RLU of the well with the primary antibody - RLU of the corresponding well without the primary antibody, everything else being equal. Note: Each dot represents an individual specimen.

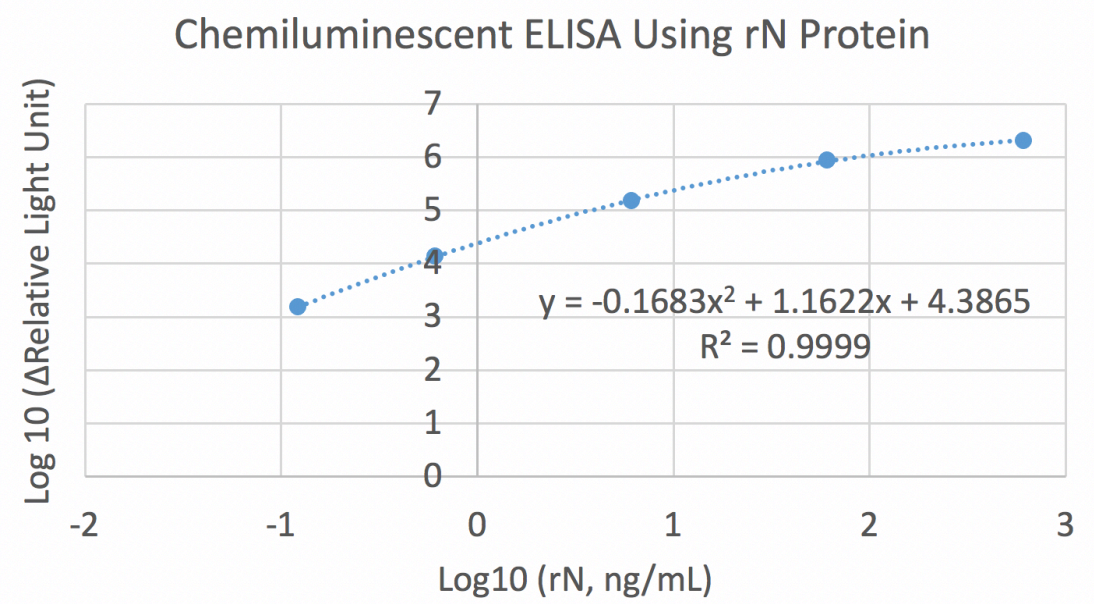

Figure 4. Chemiluminescent ELISA using recombinant nucleocapsid protein (rN). Different concentrations of rN (Cat# VPN1) lysed in the Viral Lysis Buffer (Cat# VL101) were coated on microplate wells. Rabbit monoclonal anti-N antibody (Cat# VYN7, 1:10,000) and HRP-conjugated highly cross-adsorbed goat anti-rabbit secondary antibody (Cat# 202, 1:20,000) were used to detect SARS-CoV-2 N protein. ShQ™ ECL reagents were used to generate chemiluminescent signals. RLU, relative light unit. ΔRLU = RLU of the well with the primary antibody - RLU of the corresponding well without the primary antibody, everything else being equal.

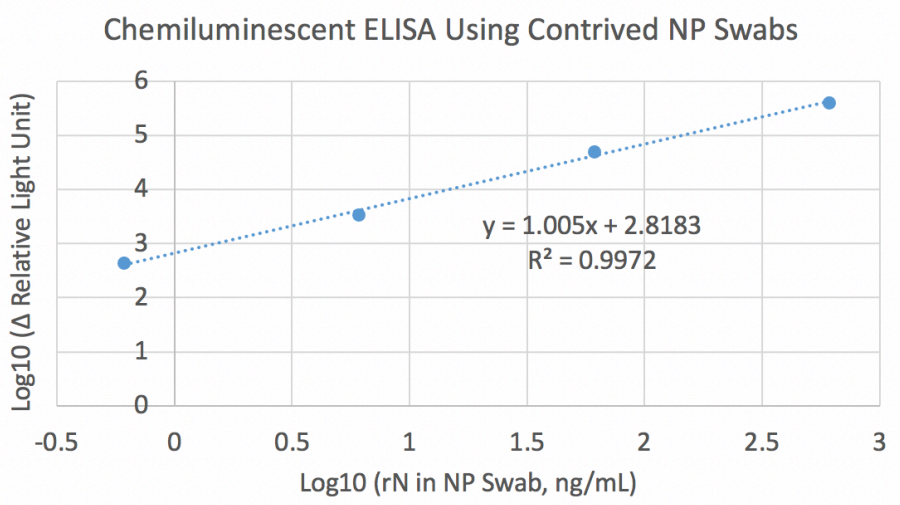

Figure 5. Chemiluminescent ELISA using contrived human nasopharyngeal (NP) swab specimens. Different concentrations of recombinant nucleocapsid protein (rN) (Cat# VPN1) were spiked in EUA RT-PCR-confirmed negative human NP swabs and lysed in the Viral Lysis Buffer (Cat# VL101). The lysates were coated on microplate wells, and rabbit monoclonal anti-N antibody (Cat# VYN7, 1:10,000) and HRP-conjugated highly cross-adsorbed goat anti-rabbit secondary antibody (Cat# 202, 1:20,000) were used to detect SARS-CoV-2 N protein. ShQ™ ECL substrates (Cat# 707) were used to generate chemiluminescent signals. RLU, relative light unit. ΔRLU = RLU of the well with the primary antibody - RLU of the corresponding well without the primary antibody, everything else being equal.

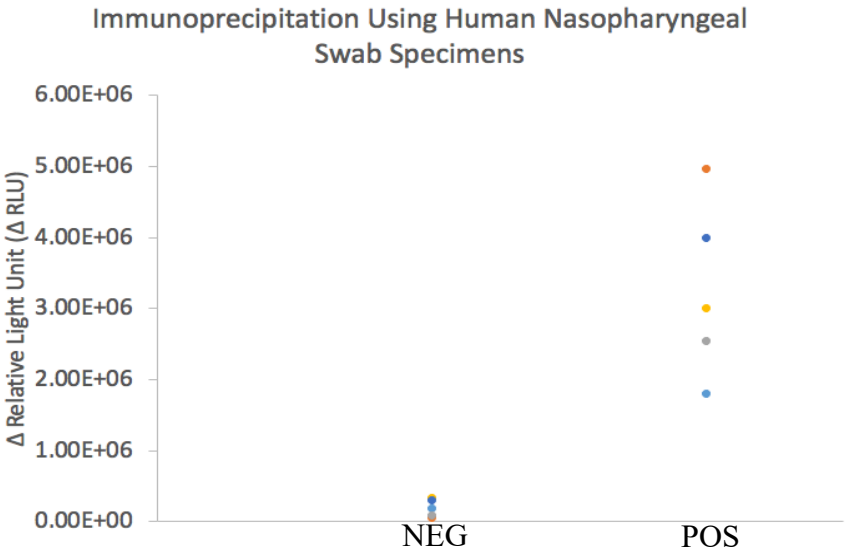

Figure 6. Immunoprecipitation (IP) using human nasopharyngeal swab specimens. Five negative (NEG) and five positive (POS) human nasopharyngeal swab specimens were precleared with the MxBind™ Protein A Magnetic Beads (Cat# VB101) and lysed in GenuIN® IP Lysis Buffer (Cat# VL202). Rabbit monoclonal anti-N antibody (Cat# VYN7, 1:50), precoated and crosslinked to MxBind™ Protein A Magnetic Beads (Cat# VB101), was incubated with the precleared nasopharyngeal swab specimen. The bound antigen was released using the Viral Lysis Buffer (Cat# VL101). ELISA was performed using rabbit monoclonal anti-N antibody (Cat# VYN7, 1:10,000) and HRP-conjugated highly cross-adsorbed goat anti-rabbit secondary antibody (Cat# 202, 1:20,000) to detect SARS-CoV-2 N protein. RLU, relative light unit. ΔRLU = RLU of the well with the primary antibody - RLU of the corresponding well without the primary antibody, everything else being equal. Note: Each dot represents an individual specimen.

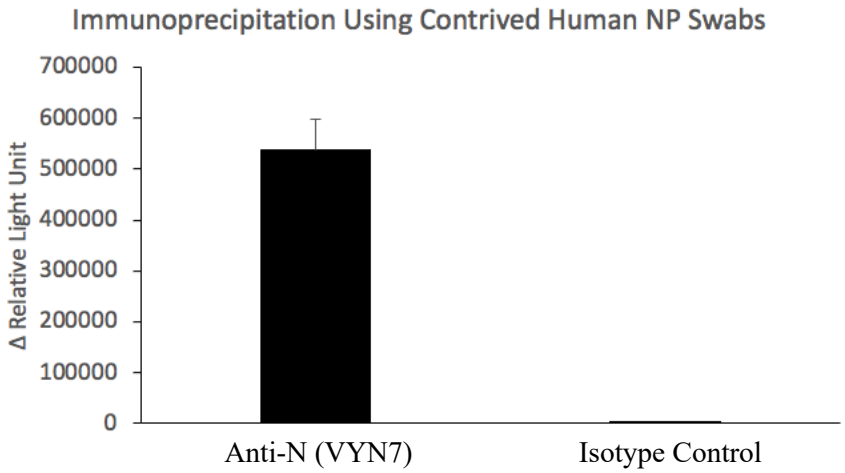

Figure 7. Immunoprecipitation (IP) using contrived human nasopharyngeal (NP) swab specimens. Recombinant N protein (rN, Cat# VPN2, 1 ng) was spiked into 2 mL of MxBind™ Protein A Magnetic Beads (Cat# VB101)-precleared, RT-PCR-confirmed negative human NP swab specimens. Rabbit monoclonal anti-N antibody (Cat# VYN7, 2 µL) was coated and cross-linked to MxBind™ Protein A Magnetic Beads (Cat# VB101), and incubated with the contrived NP swab specimens overnight at 4°C. The beads were washed and lysed in 300 µL of the Viral Lysis Buffer (Cat# VL101); the lysates were used for chemiluminescent ELISA with rabbit monoclonal anti-N antibody (Cat# VYN7, 1:10,000). HRP-conjugated highly cross-adsorbed goat anti-rabbit secondary antibody (Cat# 202, 1:20,000) was used to detect SARS-CoV-2 N protein. PiQ™ ECL (Cat# 636) substrates were used to generate chemiluminescent signals. RLU, relative light unit. ΔRLU = RLU of the well with the primary antibody - RLU of the corresponding well without the primary antibody, everything else being equal.

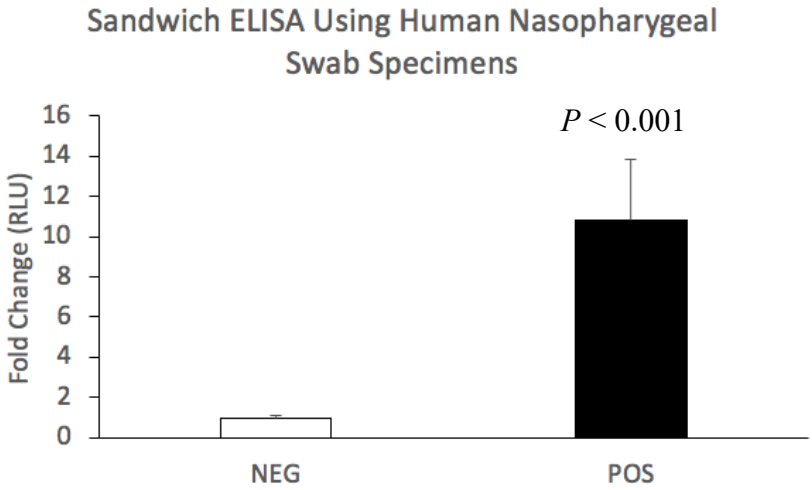

Figure 8. Chemiluminescent sandwich ELISA using human nasopharyngeal swab specimens. Five negative (NEG) and 5 positive (POS) human nasopharyngeal swab specimens were lysed in Viral Lysis Buffer (Cat# VL101). Anti-N rabbit monoclonal antibody (VYN6, 1:1,000 in 150 µL) was used to coat the microplate wells. After the binding of the lysed swab specimens, the microwells were incubated with HRP-conjugated rabbit monoclonal anti-N primary antibody (Cat# VYN7H, 1:10,000 dilution). PiQ™ ECL (Cat#639) was used to generate the chemiluminescent signals. RLU, relative light unit.

Table 1. Comparison of four monoclonal antibodies (Cat# VYN7, 5, 4, and 3) in terms of their reactivities with two rN proteins (Cat# VPN1 and 2) and two concentrations (42 and 21 $TCID_{50}$) of gamma-irradiated SARS-CoV-2 coronaviruses.

Note: rN, 0.125 ng/mL		Anti-N Monoclonal Antibodies			
		VYN7	VYN5	VYN4	VYN3
rN Protein	VPN1	135731	201369	10731	15089
	VPN2	215656	241611	154289	10606
SARS-CoV-2	42 $TCID_{50}$	4070095	1955123	790755	35934
	21 $TCID_{50}$	1665682	903818	256938	16273

Note:

1. rNs and gamma-irradiated SARS-CoV-2 were lysed in the Viral Lysis Buffer (Cat# VL101) and coated (150 µL) on microplate wells.
2. Monoclonal anti-N antibodies (1:10,000 dilution) and HRP-conjugated secondary antibodies (1:20,000) were used to detect SARS-CoV-2 N protein.
3. PiQ™ ECL (Cat# 636) substrates were used to generate chemiluminescent signals.
4. Signals were measured in delta relative light unit (ΔRLU). ΔRLU = RLU of the well with the primary antibody - RLU of the corresponding well without the primary antibody, everything else being equal.