ANTI-NUCLEOCAPSID (N) MOUSE MONOCLONAL AB #VYN3

CATALOG & PRICING

Cat. #	Conjugation	Size
VYN3-20	None	20 µL
VYN3-50		50 µL
VYN3-100		100 µL
VYN3B-20	Biotin	20 µL
VYN3B-50		50 µL
VYN3B-100		100 µL
VYN3H-20	Horseradish Peroxidase	20 µL
VYN3H-50		50 µL
VYN3H-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

- Mouse monoclonal antibody, may be used with rabbit antibodies for multiplexing immunoassays
- 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: Mouse monoclonal antibody
Clone ID: V300-M1
Species Reactivity: SARS-CoV-2
Applications Tested: Western blotting (WB), ELISA

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

IMMUNOGEN
Recombinant SARS-CoV-2 nucleocapsid protein

ISOTYPE
Mouse 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

VALIDATION DATA

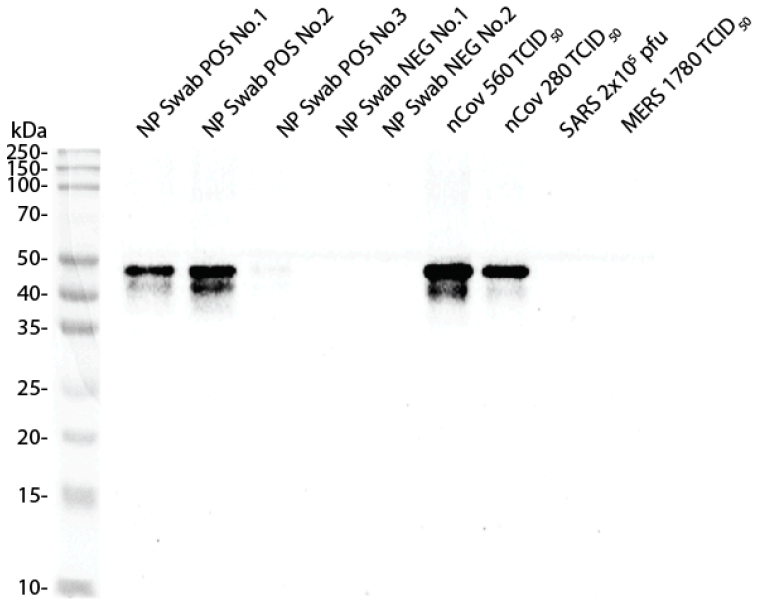

Figure 1. Antibody specificity and cross-reactivity test. Three positive and two negative human nasopharyngeal swab specimens (NP Swabs, confirmed by EUA RT-PCR), gamma-irradiated SARS-CoV-2 (nCoV), SARS-CoV (SARS), and MERS were lysed in the Viral Lysis Buffer (Cat# VL101) and blotted with mouse monoclonal anti-N antibody (Cat# VYN3, 1:2,000). HRP-conjugated goat anti-mouse IgG (Cat# 101, 1:2,000) was used as the secondary antibody. The blot was then incubated with the PiQ™ ECL substrates (Cat# 636) and imaged using a chemiluminescence digital imager. Note: 1) The NP Swab POS No.3 has a very low viral load; and 2) there was no cross-reactivity with SARS or MERS.

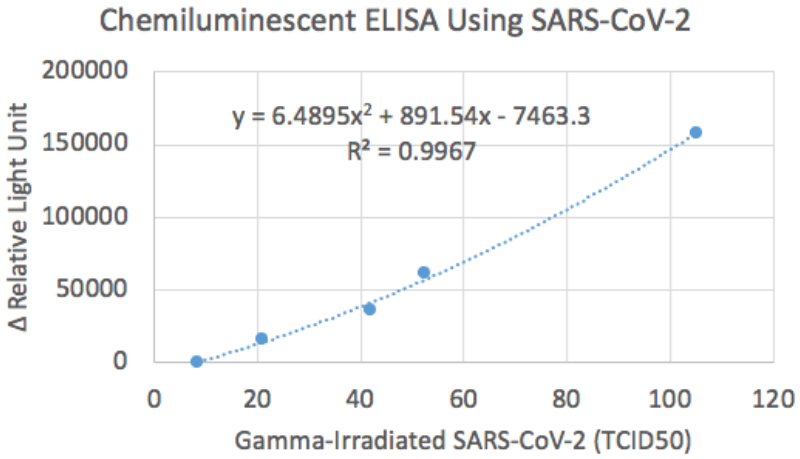

Figure 2. Chemiluminescent ELISA using SARS-CoV-2. Gamma-irradiated SARS-CoV-2 coronaviruses were lysed in the Viral Lysis Buffer (Cat# VL101) and coated (150 µL) on microplate wells. Mouse monoclonal anti-N antibody (Cat# VYN3, 1:10,000) and HRP-conjugated goat anti-mouse IgG secondary antibody (Cat# 101, 1:20,000) were used to detect SARS-CoV-2 N protein. PiQ™ ECL substrates (Cat# 636) 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.

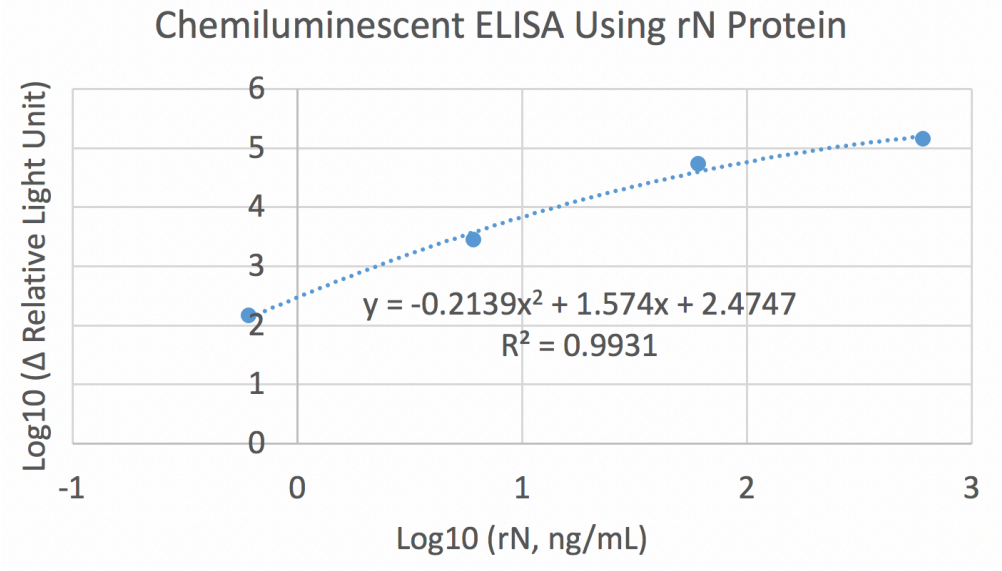

Figure 3. Chemiluminescent ELISA using recombinant nucleocapsid protein (rN). Different concentrations of rN (Cat# VPN1) lysed in the Viral Lysis Buffer (Cat# VL101) were coated on microplate wells. Mouse monoclonal anti-N antibody (Cat# VYN3, 1:10,000) and HRP-conjugated goat anti-mouse secondary antibody (Cat# 101, 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.

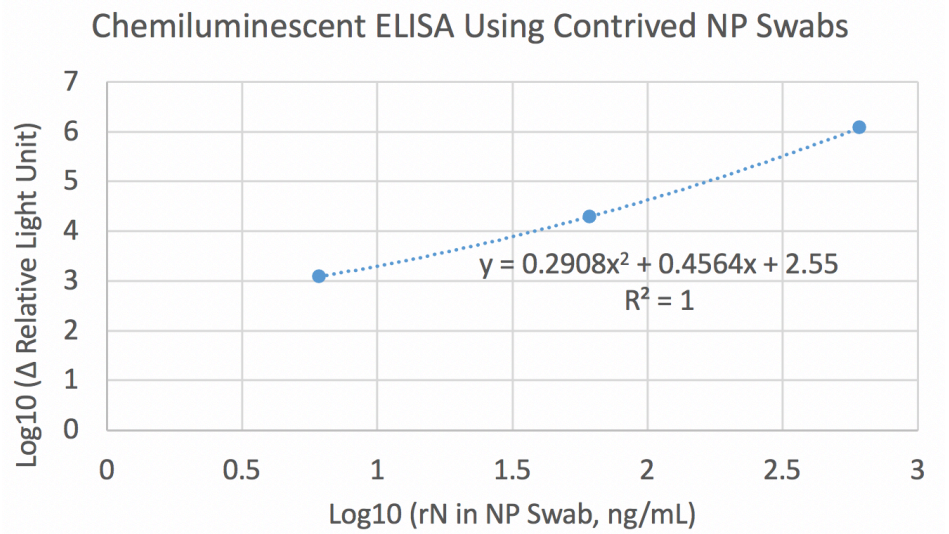

Figure 4. Chemiluminescent ELISA using contrived human nasopharyngeal (NP) swab specimens. Different concentrations of recombinant nucleocapsid protein (rN) (Cat# VPN1) were spiked in EUA RT-PCR-confirmed negative human NP swabs and lysed in the Viral Lysis Buffer (Cat# VL101). The lysates were coated on microplate wells, and mouse monoclonal anti-N antibody (Cat# VYN3, 1:10,000) and HRP-conjugated goat anti-mouse secondary antibody (Cat# 101, 1:20,000) were used to detect SARS-CoV-2 N protein. PiQ™ ECL substrates (Cat# 636) 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.

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.