

SARS-CoV-2 N protein Sandwich ELISA Kit Datasheet

For the quantitative detection of SARS-CoV-2 N protein concentrations in serum and plasma. For research use only, not for clinical diagnosis.

General Information

Catalogue Number	KE30007
Product Name	SARS-CoV-2 N protein Sandwich ELISA Kit
Species cross-reactivity	
Range (calibration Range)	375-6000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	43740575
SwissProt	PODTC9

Kit Components & Storage

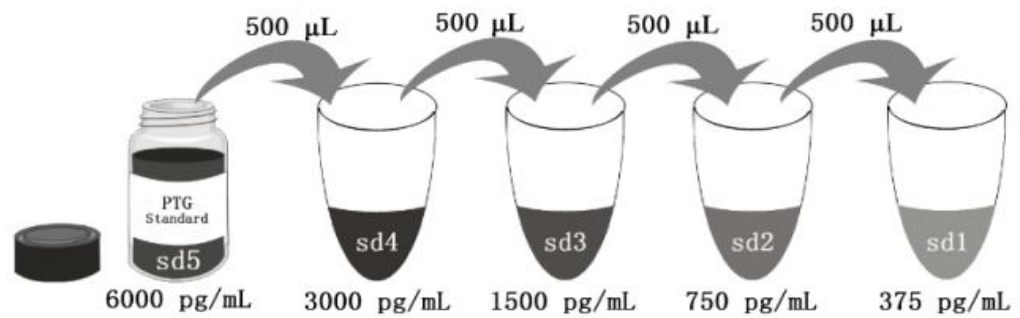
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 24000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, HRP-conjugated (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 4 - 30 mL/bottle. For Mouse/Rat serum, plasma and serum-free cell culture supernatants.	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

NB: Do not use the kit after the expiration date.

Sample Diluent PT 4 is for protein standard, serum and plasma samples.

Detection Diluent is for Detection antibody.

*Add 4 mL Sample Diluent PT 4 in protein standard. This reconstitution gives a stock solution of 6000 pg/mL.



Add # μL of Standard diluted in the previous step	—	500 μL	500 μL	500 μL	500 μL
# μL of Sample Diluent PT 4	4000 μL	500 μL	500 μL	500 μL	500 μL
	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE30007 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The SARS-CoV-2 N protein ELISA kit is to be used to detect and quantify protein levels of endogenous SARS-CoV-2 N protein. The assay recognizes human SARS-CoV-2 N protein. An antibody specific for SARS-CoV-2 N protein has been pre-coated onto the microwells. The SARS-CoV-2 N protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for SARS-CoV-2 N protein is added to detect the captured SARS-CoV-2 N protein. For signal development, horseradish peroxidase (HRP)-conjugated antibody is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

Coronaviruses are enveloped viruses with a positive-sense RNA genome and with a nucleocapsid of helical symmetry.

Coronavirus nucleoproteins localize to the cytoplasm and the nucleolus, a subnuclear structure, in both virus-infected primary cells and in cells transfected with plasmids that express N protein. Coronavirus N protein is required for coronavirus RNA synthesis and has RNA chaperone activity that may be involved in template switch. Nucleocapsid protein is a most abundant protein of coronavirus. During virion assembly, N protein binds to viral RNA and leads to formation of the helical nucleocapsid.

Nucleocapsid protein is a highly immunogenic phosphoprotein also implicated in viral genome replication and in modulating cell signaling pathways. Because of the conservation of N protein sequence and its strong immunogenicity, the N protein of coronavirus is chosen as a diagnostic tool.

Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. 1:2 dilution is recommended for the individual samples.

Safety Notes

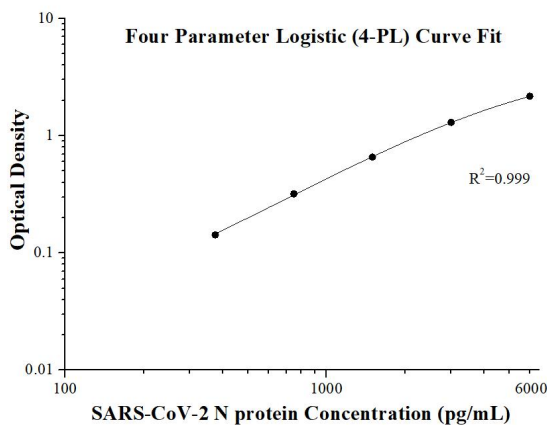
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Detecton Antibody, HRP-conjugated Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
4	Stop Solution	100 µL	0 min	Do not wash	-
5	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.031 0.035	0.033	-
375	0.19 0.185	0.188	0.143
750	0.381 0.348	0.365	0.32
1500	0.676 0.73	0.703	0.658
3000	1.384 1.303	1.344	1.299
6000	2.218 2.214	2.216	2.171

Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision					Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	20	3011.9	119.3	4.0	1	24	2936.3	76.7	2.6
2	20	693.7	38.5	5.5	2	24	682.9	30.1	4.4
3	20	327.1	22.5	6.9	3	24	326.6	22.9	7.0

Recovery

The recovery of SARS-CoV-2 N protein spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:2	122	118-128
	1:4	109	95-126

Sample Values

Sixteen serum and sixteen plasma samples from healthy volunteers were evaluated for SARS-CoV-2 N protein in this assay. All the samples measured less than the lowest standard, 375 pg/mL.

Sensitivity

The minimum detectable dose of SARS-CoV-2 N protein is 38.0 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

Linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of SARS-CoV-2 N protein in various matrices and diluted with the **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human plasma
1:2	Average% of Expected	99
	Range (%)	95-107
1:4	Average% of Expected	108
	Range (%)	103-115
1:8	Average% of Expected	107
	Range (%)	93-120

References

1. YZumla, A., Chan, J. F. W. et al. (2016). Coronaviruses-drug discovery and therapeutic options. *Nat. Rev. Drug Discov.* 15, 327–347.
2. Penghui Yang, Xiliang Wang .(2020) COVID-19: A New Challenge for Human Beings, *Cell Mol Immunol.* 17(5):555-557.