Version 01-06/20

User's Manual

SARS-CoV-2 Nucleoprotein ELISA Kit

REF **DEIASL017**

Σ **96T**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

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.

General Description

Coronaviruses are enveloped viruseswith a positive-sense RNA genome and with a nucleocapsid of helical symmetry. Coronavirus nucleoprotein slocalize 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 bindsto viral RNA and leadsto 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 coronavirusis chosen as a diagnostic tool.

Principles of Testing

SARS-CoV-2 Nucleoprotein ELISA Kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The assay recognizes human SARS-CoV2 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 samplesis 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.

Reagents And Materials Provided

1. Microplate-Antibody coated 96-well microplate (8 well × 12 strips), 1 plate
2. Protein standard-24000 pg/bottle, lyophilized, 2 bottles
3. Detection antibody, HRP-conjugated (100X)-120 µL/vial, 1 vial
4. Sample Diluent PT 4-30 mL/bottle, 1 bottle
5. Detection Diluent-30 mL/bottle, 1 bottle
6. Wash Buffer Concentrate (20X)-30 mL/bottle, 1 bottle
7. Tetramethylbenzidine Substrate (TMB)-12 mL/bottle, 1 bottle
8. Stop Solution-12 mL/bottle, 1 bottle
9. Plate Cover Seals, 3 pieces

Storage

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.

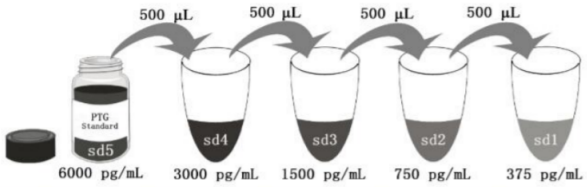
Specimen Collection And Preparation

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.

Reagent Preparation

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"	"sd5"	"sd4"	"sd3"

Assay Procedure

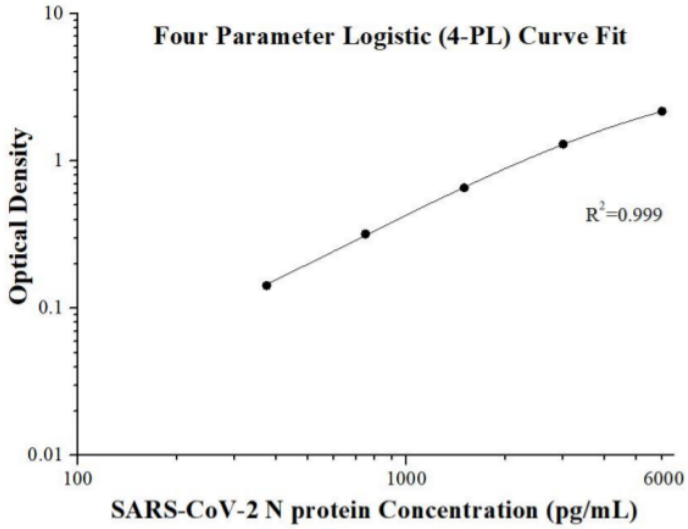
Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Deteciton 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.				

Typical Standard Curve

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



Reference 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.

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.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	3011.9	119.3	4.0
2	20	693.7	38.5	5.5
3	20	327.1	22.5	6.9

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

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	2936.3	76.7	2.6
2	24	682.9	30.1	4.4
3	24	326.6	22.9	7.0

Detection Range

375-6000 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.

Specificity

SARS-CoV-2 Nucleoprotein

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 valueswithin 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

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

Precautions

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.

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 NewChallenge for Human Beings, Cell Mol Immunol. 17(5):555-557.