Version 14-05/24

User's Manual

SARS-CoV-2 N ELISA Kit

REF **DEIASL017**

Σ **96T**

RUO

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.

Creative Diagnostics

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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 concentrations in in serum and plasma. For research use noly, not for clinical diagnosis.

General Description

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 coronavirusis chosen as a diagnostic tool.

Principles of Testing

This is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). 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.

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 (100×) - 120 µL/vial, 1 vial
4. Sample Diluent - 30 mL/bottle, 1 bottle
5. Detection Diluent - 30 mL/bottle, 1 bottle
6. Wash Buffer Concentrate (20×) - 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

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

Detection Diluent is for Detection antibody.

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

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.

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

Specimen Collection And Preparation

Please Note:

1. All biological samples should be carried out in accordance with institutional safety guidelines and restrictions.
2. Please run a preliminary experiment to optimize the sample dilution and to check if the sample is within the assay range.
3. Do not use turbid or grossly hemolyzed samples. Mix thawed samples thoroughly before starting the ELISA assay.

A. Serum

Allow blood samples to clot for 30 minutes prior to centrifugation for 15 minutes at 1000 × g. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

B. Plasma

Use EDTA or heparin as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

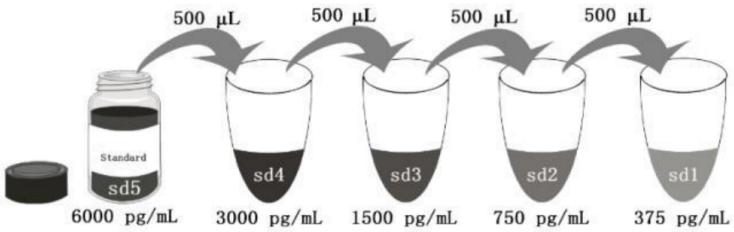
The serum or plasma samples may require proper dilution to fall within the range of the assay. 1:2 dilution is recommended for the individual samples.

Reagent Preparation

A. Standard

Always prepare a fresh set of standards for every use. It is recommended to run the experiment in duplicate at a minimum.

1. Reconstitute the Standard with 4mL Sample Diluent (label it "sd5"). This reconstitution gives a stock solution with an assumed concentration of 6000 pg/mL. Use Standards within 30 minutes of reconstitution.
2. Perform serial dilutions of standards as described in the following figure; i.e., carry over 500 µL of standard from last dilution into a tube containing 500 µL fresh Sample Diluent. Mix thoroughly between steps.
3. Use Sample Diluent as your blank/zero standard; label it "sd0".



B. Detection antibody, HRP-conjugated

1. Dilute 100X Detection antibody, HRP-conjugated 1:100 using Detection Diluent prior to assay.

   Suggested 1:100 dilution: 10 µL 100X Detection antibody, HRP-conjugated + 990 µL Detection Diluent.

   Please Note:

   • Centrifuge the 100X Detection antibody, HRP-conjugated solution for a few seconds prior to use.

C. Wash Buffer

1. Allow the 20X Wash Buffer to reach room temperature before use. Dilute entire 30 mL of 20X Wash Buffer concentrate with 570 mL deionized, distilled water. If crystals remain in the concentrate, warm to 37°C and mix gently until the crystals have dissolved completely. Store at 2–8°C.

Assay Procedure

1. Prepare all reagents, samples, and working standards as instructed.
2. Take out the required number of microplate strips and place the microwells in the strip holder. In the meantime, return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used as soon as possible.
3. Add 100 µL of each standard and sample to the appropriate wells. (Make sure sample addition is uninterrupted and completed within 5 to 10 minutes).
4. Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 120 min at 37°C in a humid environment.
5. Wash wells:

   i. Gently remove the cover seal.

   ii. Discard the liquid from wells by aspirating or decanting.

   iii. Remove any residual solution by tapping the plate a few times on fresh paper towels.

   iv. Wash 4 times with 1X Wash Buffer, using at least 300 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

6. Add 100 µL of 1X Detection antibody, HRP-conjugated solution to each well. Seal plate with cover seal and incubate for 40 min at 37°C in a humid environment. Repeat the washes in step 5.
7. Signal development: Add 100 µL of TMB substrate solution to each well. Incubate for 15 to 20 minutes at 37°C in the dark. A positive reaction will be indicated by the color blue. (Longer incubation times are recommended in the event of the blue color appearing too pale). **Do not wash.**
8. Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of

the TMB substrate. Mix by tapping the side of the plate gently. The color will change from blue to yellow. **Do not wash.**

Please Note:

• Avoid any skin and eye contact with Stop solution.

9. Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm). The absorbance should be measurable immediately after adding Stop solution. DO NOT exceed 5 minutes.

Calculation

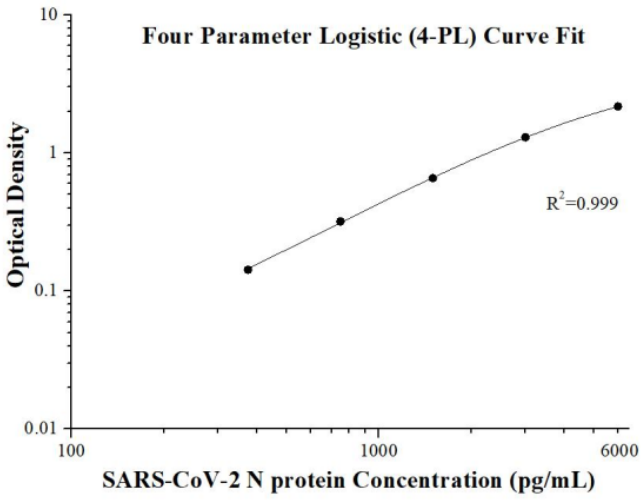
Average the duplicate readings for each standard and sample and subtract the average zero standard absorbance (obtained from the average of the "sd0" readings). The best-fit standard curve can be determined by regression analysis using four-parameter logistic curve fit (4-PL). As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best-fit curve through the points on the graph. The data may be linearized by plotting the log of the Standard concentrations versus the log of the OD readouts. The best-fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Please Note:

• If the samples have been diluted, the OD readout from the standard curve must be multiplied by the corresponding dilution factor.

Typical Standard Curve

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

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.

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				
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				
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.

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

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.

Limitations

1. Do not use the kit after the expiration date.
2. This product is for research use only and is not for use in human or animal therapeutic or diagnostic use.
3. Strictly adhere to the instructions in this manual. Do not mix or substitute reagents or materials from other kit lots or other sources.
4. Samples may require further dilution if the readout values are higher than the highest standard OD reading. Dilute the samples with Sample Diluent and ensure that their absorbance reading falls within those generated by the standards.
5. Perform the assay in replicates as errors can be introduced by the operator, such as poor pipetting technique, wash technique, or incorrect incubation times or temperatures

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.

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