（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !）

Catalog No: E-EL-E604

Size: 96T/48T/24T/96T*5

Elabscience® SARS-CoV-2 Nucleocapsid Protein ELISA Kit

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com

Please refer to specific expiry date from label outside of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

Intended use

This ELISA kit applies to the in vitro quantitative determination of SARS-CoV-2 Nucleocapsid protein (N protein) concentrations in human serum, plasma and other appropriate biological fluids.

Character

Item	
Sensitivity	0.12 ng/mL
Detection Range	0.39-25ng/mL
Specificity	This kit recognizes SARS-CoV-2 N protein in the tested samples. No significant cross-reactivity or interference between N protein and analogues was observed
Repeatability	Coefficient of variation is < 10%

Test principle

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to SARS-CoV-2 N protein. Samples (or Standards) and biotinylated detection antibody specific for SARS-CoV-2 N protein are added to the micro ELISA plate wells. SARS-CoV-2 N protein would combine with the specific antibody. Then Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain SARS-CoV-2 N protein, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional to the concentration of SARS-CoV-2 N protein. You can calculate the concentration of SARS-CoV-2 N protein in the samples by comparing the OD of the samples to the standard curve.

Kit components & Storage

An unopened kit can be stored at 2-8℃ for 12 months. After opening, store the items separately according to the following conditions.

Item	Specifications	Storage
Micro ELISA Plate (Dismountable)	96T: 8 wells ×12 strips 48T: 8 wells ×6 strips 24T: 8 wells ×3 strips 96T*5: 5 plates, 96T	-20℃, 12 months
Reference Standard	96T: 2 vials 48T/24T: 1 vial 96T*5: 10 vials	
Concentrated Biotinylated Detection Ab(100×)	96T: 1 vial, 120 μL 48T/24T: 1 vial, 60 μL 96T*5: 5 vials, 120 μL	
Concentrated HRP Conjugate (100×)	96T: 1 vial, 120 μL 48T/24T: 1 vial, 60 μL 96T*5: 5 vials, 120 μL	-20℃(Protect from light), 12 months
Reference Standard & Sample Diluent	96T/48T/24T: 2 vials, 20 mL 96T*5: 10 vials, 20 mL	2-8℃, 12 months
Biotinylated Detection Ab Diluent	96T/48T/24T: 1 vial, 14 mL 96T*5: 5 vials, 14 mL	
HRP Conjugate Diluent	96T/48T/24T: 1 vial, 14 mL 96T*5: 5 vials, 14 mL	
Concentrated Wash Buffer(25×)	96T/48T/24T: 1 vial, 30 mL 96T*5: 5 vials, 30 mL	
Substrate Reagent	96T/48T/24T: 1 vial, 10 mL 96T*5: 5 vials, 10 mL	2-8℃(Protect from light)
Stop Solution	96T/48T/24T: 1 vial, 10 mL 96T*5: 5 vials, 10 mL	2-8℃
Plate Sealer	96T/48T/24T: 5 pieces 96T*5: 25 pieces	
Product Description	1 copy	

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution. The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

Other supplies required

Microplate reader with 450nm wavelength filter
High-precision transfer pipette, EP tubes and disposable pipette tips
Incubator capable of maintaining 37℃
Deionized or distilled water
Absorbent paper
Loading slot for Wash Buffer

Note

1. Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
2. A freshly opened ELISA Plate may appear to have a water-like substance, which is normal and will not have any impact on the experimental results. Return the unused wells to the foil pouch provided in the kit, store it according to the conditions suggested in the above table.
3. Do not reuse the reconstituted standard, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab (100×) and other stock solutions should be stored according to the storage conditions in the above table.
4. The microplate reader should be able to be installed with a filter that can detect the wave length at 450±2 nm. The optical density should be within 0-3.5. Follow the Instructions of the Microplate Reader for set-up and preheat it for 15 min before OD measurement.
5. Do not mix or use components with those from other lots.
6. Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.

Sample collection

(More detailed information please view our website:
http://www.elabscience.com/List-detail-253.html)
Serum: Allow samples to clot for 1 hours at room temperature or overnight at 2-8℃before centrifugation for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.
Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8℃ within 30 min of collection. Collect the supernatant to carry out the assay.
Cell lysates: For adherent cells, gently wash the cells with moderate amount of

pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000×g. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1×10^6 cells, add 150-250 μL of pre-cooled PBS to keep the cells suspended. Repeat the freeze-thaw process several times or use an ultrasonic cell disrupter until the cells are fully lysed. Centrifuge for 10min at 1500×g at 2-8℃. Remove the cell fragments, collect the supernatant to carry out the assay.

Cell culture supernatant or other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

Note for sample

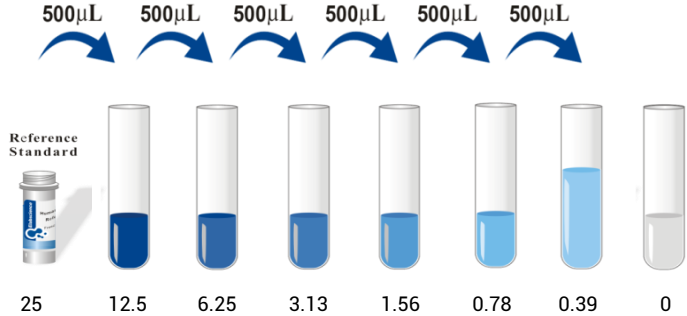
1. Tubes for blood collection should be disposable and be non-endotoxin. Samples with high hemolysis or much lipid are not suitable for ELISA assay.
2. Samples should be assayed within 7 days when stored at 2-8℃, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.
3. Please predict the concentration before assay. For tested samples, a preliminary experiment is strongly suggested to determine the optimal dilution factors for their particular experiments.
4. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
5. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance.
6. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

Reagent preparation

1. Bring all reagents to room temperature (18-25℃) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
2. **Wash Buffer:** Dilute 30mL of Concentrated Wash Buffer with 720mL of deionized or distilled water to prepare 750mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 25ng/mL(or add

1.0mL of Reference Standard &Sample Diluent, let it stand for 1-2 min and then mix it thoroughly with a vortex meter of low speed. Bubbles generated during vortex could be removed by centrifuging at a relatively low speed). Then make serial dilutions as needed. The recommended dilution gradient is as follows: 25、12.5、6.25、3.13、1.56、0.78、0.39、0ng/mL.

Dilution method: Take 7 EP tubes, add 500μL of Reference Standard & Sample Diluent to each tube. Pipette 500μL of the 25ng/mL working solution to the first tube and mix up to produce a 12.5ng/mL working solution. Pipette 500μL of the solution from the former tube into the latter one according to this step. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.



4. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (100μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated Biotinylated Detection Ab at 800×g for 1 min, then dilute the 100× Concentrated Biotinylated Detection Ab to 1× working solution with Biotinylated Detection Ab Diluent(Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent= 1: 99).
5. **HRP Conjugate working solution:** HRP Conjugate is HRP conjugated avidin. Calculate the required amount before the experiment (100μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800×g for 1 min, then dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent(Concentrated HRP Conjugate: HRP Conjugate Diluent= 1: 99).

Assay procedure

1. Determine wells for diluted standard, blank and sample. Add 100μL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate). Cover

the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Decant the liquid from each well, do not wash. Immediately add 100μL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37°C.
3. Decant the solution from each well，add 350μL of **wash buffer** to each well. Soak for 1-2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.
4. Add 100μL of **HRP Conjugate working solution** to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.
5. Decant the solution from each well, repeat the wash process for 5 times as conducted in step 3.
6. Add 90μL of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min. Preheat the Microplate Reader for about 15 min before OD measurement.
7. Add 50μLof **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Calculation of results

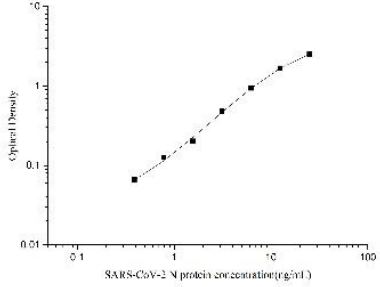
Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.

Concentration(ng/mL)	25	12.5	6.25	3.13	1.56	0.78	0.39	0
OD	2.6	1.746	1.012	0.55	0.274	0.198	0.138	0.072
Corrected OD	2.528	1.674	0.94	0.478	0.202	0.126	0.066	-



Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level SARS-CoV-2 N protein were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level SARS-CoV-2 N protein were tested on 3 different plates, 20 replicates in each plate.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean(ng/mL)	1.21	3.04	10.9	1.31	3.21	11.08
Standard deviation	0.06	0.13	0.54	0.09	0.16	0.59
CV (%)	4.96	4.28	4.95	6.87	4.98	5.32

Recovery

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

Sample Type	Range (%)	Average Recovery (%)
Serum (n=5)	82-115	97
EDTA plasma (n=5)	85-98	87

Linearity

Samples were spiked with high concentrations of SARS-CoV-2 N protein and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

		Serum (n=5)	EDTA plasma (n=5)
1:2	Range (%)	85-102	88-110
	Average (%)	94	101
1:4	Range (%)	91-119	88-99
	Average (%)	103	93
1:8	Range (%)	82-107	88-106
	Average (%)	94	96
1:16	Range (%)	88-118	85-117
	Average (%)	108	101

SUMMARY

1. Add 100μL standard or sample to each well. Incubate for 90 min at

2. Remove the liquid. Add 100μL Biotinylated Detection Ab. Incubate for 1 hour at 37℃.

3. Aspirate and wash 3 times.

4. Add 100μL HRP Conjugate. Incubate for 30 min at 37℃.

5. Aspirate and wash 5 times.

6. Add 90μL Substrate Reagent. Incubate for 15 min at 37℃.

7. Add 50μL Stop Solution. Read at 450 nm immediately.

8. Calculation of results.

Troubleshooting

If the results are not good enough, please take pictures and save the experimental data in time. Keep the used plate and remaining reagents. Then contact our technical support to solve the problem. Meanwhile, you could also refer to the following materials:

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing.
	Wells are not completely aspirated	Completely aspirate wells in between steps.
Low signal	Insufficient incubation time	Ensure sufficient incubation time.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation.
	Improper dilution	
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB, rapid coloring.
Deep color but low value	Plate reader setting is not optimal	Verify the wavelength and filter setting on the Microplate reader.
Large CV	Inaccurate pipetting	Check pipettes
High background	Concentration of target protein is too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions.
	Stop solution is not added	Stop solution should be added to each well before measurement.

Declaration

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.
3. The final experimental results will be closely related to the validity of products, operational skills of the operators, the experimental environments and so on. We are only responsible for the kit itself, but not for the samples consumed during the assay. The users should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
4. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions.
5. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
6. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
7. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipment, and so on. Intra-assay variance among kits from different batches might arise from the above reasons, too.
8. Kits from different manufacturers or other methods for testing the same analyte could bring out inconsistent results, since we haven't compared our products with those from other manufacturers.
9. The kit is designed for research use only, we will not be responsible for any issues if the kit is applied in clinical diagnosis or any other related procedures.