



Technical Manual

SARS-CoV-2 Nucleocapsid Protein ELISA Kit

- **Catalogue Code: HUES03621**
- **Sandwich ELISA Kit**
- **Research Use Only**

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Intended Use

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

Test Principle

This 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.

Key Featured and Sample Types

- Sensitivity: 0.12ng/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%.

SUMMARY

1. Add 100µL standard or sample to each well. Incubate for 90 min at 37°C.
2. Remove the liquid. Add 100µL Biotinylated Detection Ab. Incubate for 1 hour at 37°C.
3. Aspirate and wash 3 times.
4. Add 100µL HRP Conjugate. Incubate for 30 min at 37°C.
5. Aspirate and wash 5 times.
6. Add 90µL Substrate Reagent. Incubate for 15 min at 37°C.
7. Add 50µL Stop Solution. Read at 450 nm immediately.
8. Calculation of results

Kit Contents

Product	Size	Cat. Code
SARS-CoV-2 Nucleocapsid ELISA Kit	96 assays	HUES03621

An unopened kit can be stored at 2-8°C for 1 month. If the kit is not used within 1 month, store the items separately according to the following conditions once the kit is received.

Item	24T	96T	Storage
Micro ELISA Plate (Dismountable)	8 wells x 3 strips	8 wells x 12 strips	-20°C, 6 months
Reference Standard	1 vial	2 vials	
Concentrated Biotinylated Detection Ab (100×)	1 vial, 60 µL	1 vial, 120 µL	
Concentrated HRP Conjugate (100×)	1 vial, 60 µL	1 vial, 120 µL	-20°C (shading light), 6 months
Reference Standard & Sample Diluent	1 vial, 20 mL	1 vial, 20 mL	4°C, 6 months
Biotinylated Detection Ab Diluent	1 vial, 14 mL	1 vial, 14 mL	
HRP Conjugate Diluent	1 vial, 14 mL	1 vial, 14 mL	
Concentrated Wash Buffer (25×)	1 vial, 30 mL	1 vial, 30 mL	
Substrate Reagent	1 vial, 10 mL	1 vial, 10 mL	4°C (shading light)
Stop Solution	1 vial, 10 mL	1 vial, 10 mL	4°C
Plate Sealer	5 pieces	5 pieces	
Product Description	1 copy	1 copy	

Notes: 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).

Additional Materials required

1. Microplate reader with 450 nm wavelength filter
2. High-precision transfer pipette, EP tubes and disposable pipette tips
3. Incubator capable of maintaining 37°C
4. Deionized or distilled water
5. Absorbent paper
6. Loading slot for Wash Buffer

Additional Notes:

1. Please wear lab coats, eye goggles and latex gloves for protection. Perform the experiment following the national safety guidelines for biological laboratories, especially when using blood samples or other bodily fluids.
2. A freshly opened ELISA Plate may appear to have a water-like substance, this 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 have a 450(±10 nm) filter installed and a detector that can detect this wavelength. 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 from other lots.
6. Change pipette tips in between adding standards, sample additions and reagent additions. Also, use separate reservoirs for each reagent.

Sample collection and preparation

Serum: Allow samples to clot for 2 hour at room temperature or overnight at 2-8°C before centrifugation for 20 min at 1000×g at 2-8°C . Collect the supernatant to carry out the assay.

Plasma: Collect plasma using EDTA or heparin sodium as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2- 8°C 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°C. 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°C. Collect the supernatant to carry out the assay.

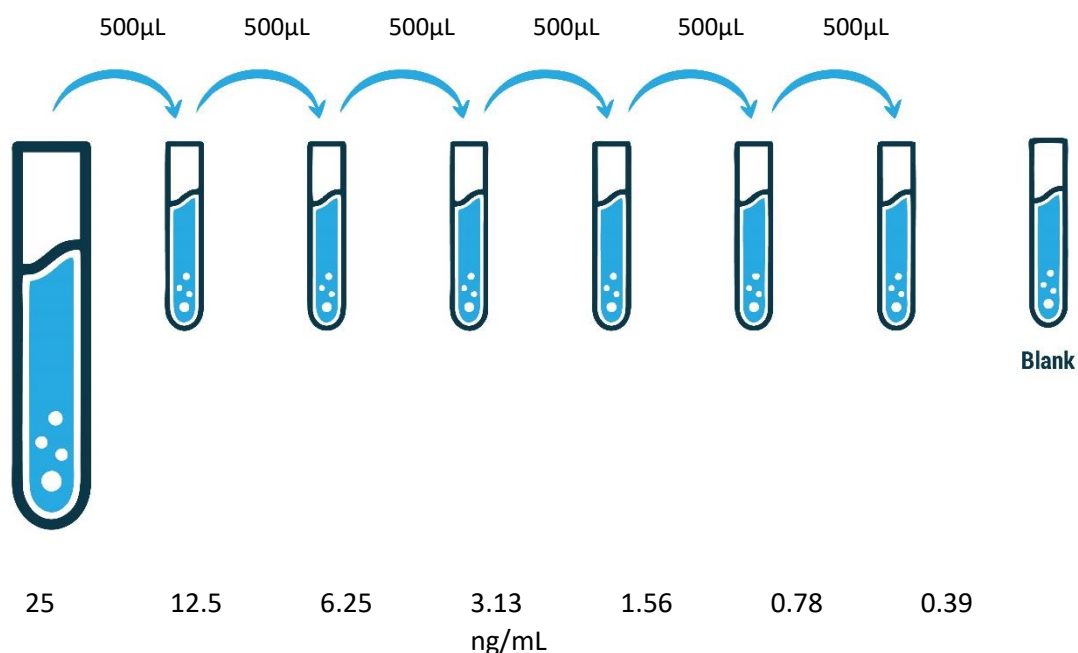
Notes :

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°C, otherwise samples must be divided up and stored at -20°C (≤1 month) or -80°C (≤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. All reagents must be brought to room temperature (18-25°C) at least 30 min 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°C 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:** 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°C. **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 50µL of **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.

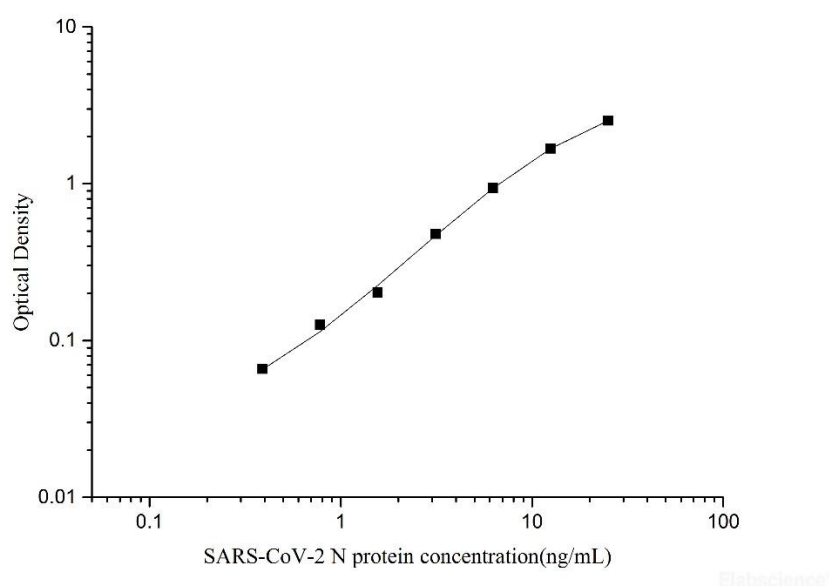
Calculation of Results

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)	O.D	Corrected
25	2.6	2.528
12.5	1.746	1.674
6.25	1.012	0.94
3.13	0.55	0.478
1.56	0.274	0.202
0.78	0.198	0.126
0.39	0.138	0.066
0	0.072	--



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.

Sample	Intra-assay Precision			Inter-assay Precision		
	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
C V (%)	4.69	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 (%)	88-107	88-106
	Average (%)	94	96
1:16	Range (%)	88-118	85-117
	Average (%)	108	101

Important General Notes:

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Centrifuge the standard vial and ensure contents are dissolved thoroughly.
	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 color change.
Deep color but low value	Plate reader setting is not optimal	Verify the wavelength and filter setting on the Microplate reader.
		Pre-heat 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 washing procedure. 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.





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