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User Manual

The manual may be updated as a result of continuous improvements.

Please refer to the hard copy manual included in the kit for your experiment.

SARS-CoV-2 Neutralization Antibody ELISA Kit(Quantitative)

Catalog No: **EKE608**

96T/24T/96T*5

This kit is for scientific research use only. Not for use in diagnostic procedures.

Intended use

This ELISA kit applies to the in vitro semi-quantitative determination of Neutralization antibodies against SARS-CoV-2 in human serum or plasma.

Specification

- Sensitivity: 9.38 ng/mL
- Detection Range: 15.63-500 ng/mL
- Specificity: This kit recognizes SARS-CoV-2 Neutralization Antibody in samples. No significant cross-reactivity or interference between SARS-CoV-2 Neutralization Antibody and analogues was observed
- Repeatability: Coefficient of variation is < 10%.

Test principle

This Test kit uses Competitive-ELISA as the method to semi-quantitatively detect the Anti-SARS-CoV-2 Neutralization Antibody in the sample. The micro ELISA plate provided in this kit is pre-coated with recombinant human ACE2. During the reaction, the SARS-CoV-2 Neutralization Antibody in the pretreated samples or standards/controls competes with a fixed amount of human ACE2 on the solid phase supporter for sites on the Horseradish peroxidase (HRP) conjugated recombinant SARS-CoV-2 RBD fragment (HRP-RBD). After 37°C incubation, the unbound HRP-RBD as well as any HRP-RBD bound to non-Neutralization antibody will be captured on the plate and eventually form the ACE2-RBD-HRP complex, while the circulating neutralization antibodies HRP-RBD complexes remain in the supernatant and are removed during washing. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm $\pm$ 2 nm. The concentration of SARS-CoV-2 Neutralization Antibody in the samples is then determined by comparing the OD of the samples to the standard curve.

Kit components & Storage

An unopened kit can be stored for 6 months at 2-8°C. After opening, store the items separately according to the following conditions.

Item	Specifications	Storage
Micro ELISA Plate (Dismountable)	24T: 8 wells ×3 strips 96T: 8 wells ×12 strips 96T*5: 5 plates, 96T	2-8°C, 1 week
Reference Standard	24T: 1 vial 96T: 2 vials 96T*5: 10 vials	2-8°C, 6 months
Positive Control	24T: 1 vial 96T: 2 vials 96T*5: 10 vials	
Negative Control	96T: 1 vial, 120 µL 48T/24T: 1 vial, 60 µL 96T*5: 5 vials, 120 µL	
Concentrated HRP Conjugate (100×)	96T/48T/24T: 1 vial, 20 mL 96T*5: 5 vials, 20 mL	2-8°C(protect from light), 6 months
Reference Standard & Sample Diluent	96T/48T/24T: 1 vial, 14 mL 96T*5: 5 vials, 14 mL	2-8°C, 6 months
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°C(protect from light)
Stop Solution	96T/48T/24T: 1 vial, 10 mL 96T*5: 5 vials, 10 mL	2-8°C, 6 months
Plate Sealer	96T/48T/24T: 5 pieces 96T*5: 25 pieces	
Product Description	1 copy	
Certificate of Analysis	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 450 nm wavelength filter
High-precision transfer pipette, EP tubes and disposable pipette tips
Incubator capable of maintaining 37 °C
Deionized or distilled water
Absorbent paper
Loading slot for Wash Buffer

Note

1. For research use only.
2. 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.
3. A freshly opened ELISA plate may appear 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 and store according to the conditions suggested in the above table.
4. The microplate reader should be able to be installed with a filter that can detect the wavelength at 450 ± 10 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 substitute reagents with those from other lots or sources.
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.
7. The kit should not be used beyond the expiration date on the kit label.

Sample collection

(More detailed information please view our website: <http://www.elabs-science.com/Listdetail-253.html>)

Serum: Allow samples to clot for 1 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-Na₂ 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.

Note for sample

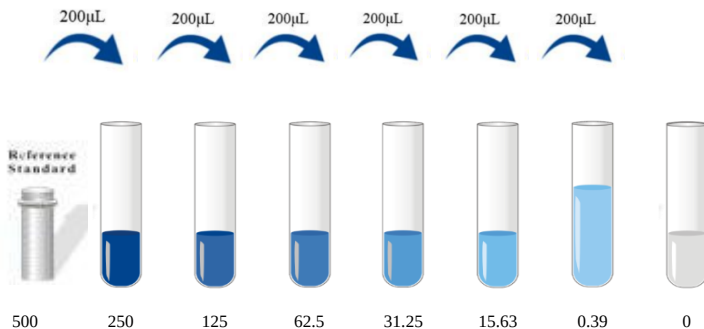
1. Tubes for blood collection should be disposable and be non-endotoxin. Severe hemolysis, lipoid, or turbidity samples should not be used.
2. Handle all serum and plasma as if capable of transmitting infectious agents.
3. Samples should be assayed within 3 days when stored at 2-8°C, otherwise samples must be divided up and stored at -20°C ($\leq$ 1 month) or -80°C ($\leq$ 3 months). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates. Frozen samples must be mixed well and brought to room temperature before testing.

Reagent preparation

1. Bring all reagents to room temperature (18-25°C) before use. If the kit will not be used 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 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL 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 $10,000 \times g$ for 1 min. Add 0.4 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 500 ng/mL (or add 0.4 mL 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: 500、250、125、62.5、31.25、15.63、0 ng/mL. Dilution method: Take 6 EP tubes, add 200 μ L of Reference Standard & Sample Diluent to each tube. Pipette 200 μ L of the 500 ng/mL working solution to the first tube and mix up to produce a 250 ng/mL working solution. Pipette 200 μ L 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. HRP Conjugate working solution: HRP Conjugate is HRP conjugated RBD. Calculate the required amount before the experiment (50 μ L/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800 $\times$ g for 1 min, then dilute the 100 $\times$ Concentrated HRP Conjugate to 1 $\times$ working solution with HRP Conjugated Diluent (Concentrated HRP Conjugate: HRP Conjugated Diluent = 1: 99). Note: The HRP Conjugate working solution should be stored at 2-8°C and used within 1 day.
5. Samples: Dilute the tested serum or plasma at 10 fold by using the Reference Standard & Sample Diluent, mix thoroughly.
6. Positive control : Dissolve Positive Control with 0.2 mL Reference Standard & Sample Diluent.
7. Negative control: Dissolve Negative Control with 0.5 mL Reference Standard & Sample Diluent.
8. Dissolved standard, pre-treated Samples and Controls should be stored at 2-8°C and used within 1 day

Assay procedure

1. Determine wells for diluted Standard, Blank, Positive/Negative Controls and Samples. Add 50 μ L each dilution of standard, pre-treated Samples and Controls into the appropriate wells (It is recommended that all Samples, Standards and Controls be assayed in duplicate). Immediately add 50 μ L of HRP conjugated working solution to each well. Cover the plate with the sealer provided in the kit. Incubate for 60 min at 37°C. Note: solutions should be added to the bottom of the micro TEST plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Decant the solution from each well, add 350 μ L of wash buffer to each well. Soak for 30-60 seconds 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.
3. Add 90 μ L of Substrate Reagent to each well. Cover the plate with a new 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 30 min. Preheat the Microplate Reader for about 15 min before OD measurement.
4. Add 50 μ L of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
5. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

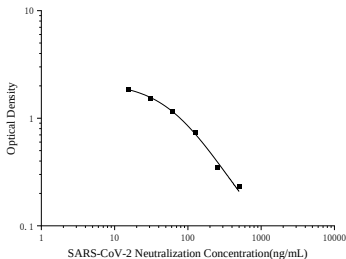
Calculation of results

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 data is provided below for reference only.

Concentration (ng/mL)	500	250	125	62.5	31.25	15.63	0
OD	0.231	0.352	0.737	1.165	1.519	1.853	2.260



Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, midrange and high level SARS -CoV-2 Nab were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, midrange and high level SARS -CoV-2 Nab were tested on 3 different plates, 20 replicates in each plate, respectively

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	17.74	110.98	413.33	18.21	117.00	424.56
Standard deviation	0.84	7.41	34.77	1.28	9.77	28.65
CV (%)	4.73	6.68	8.41	7.05	8.35	6.75

Recovery

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

Sample Type	Range (%)	Average Recovery (%)
Serum (n=8)	90-101	94
EDTA plasma (n=8)	95-107	101
Heparin Plasma (n=8)	92-106	98

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=10)	EDTA plasma (n=10)	Heparin Plasma (n=10)
1:9	Range (%)	78-101	89-112	79-103
	Average (%)	91	101	92
1:18	Range (%)	82-97	80-101	82-115
	Average (%)	88	91	98
1:36	Range (%)	81-103	84-121	91-99
	Average (%)	91	103	95
1:72	Range (%)	79-95	91-108	86-107
	Average (%)	87	98	96

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.

SUMMARY

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

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.
10. Valid period: 12 months.