Anti-SARS-CoV-2 ELISA
IgG

Insert

1. Intended Use

This Anti-SARS-CoV-2 ELISA (IgG) kit has been designed for the detection and the quantitative and qualitative determination of specific IgG antibodies against SARS-CoV-2 in plasma. This kit is intended for Research Use only and is not for use in diagnostic or therapeutic procedures.

2. Background

Coronavirus disease 2019 (COVID-19) is caused by a new coronavirus in December 2019. Because it is a new virus, scientists are learning more each day. Although most people who have COVID-19 have mild symptoms, COVID-19 can also cause severe illness and even death. Some groups, including older adults and people who have certain underlying medical conditions, are at increased risk of severe illness.

2.1 Principle of the test

The Anti-SARS-CoV-2 ELISA (IgG) kit is an enzyme immunoassay (EIA). SARS-CoV-2 antigen is bound on the surface of the microtiter strips. Diluted patient plasma or standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the plasma and the immobilized SARS-CoV-2 antigen takes place. After a one hour incubation at 37°C, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then reconstituted conjugate is added and incubated for 1 hour at 37°C. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 30 minutes. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.
The microwell plate (Cat No. ATGCH001) is coated with the recombinant S1 Spike protein (Manufacturer: NKMAX Co., Ltd., CAT NO. ATGP3961) expressed in HEK293.

2.2 Time required for performing the test

Total time to manually perform the test on one full plate: Approximately 3 hours. For each extra plate run in parallel, 10~15 minutes of hands-on time are additionally required.

3. Reagent and Storage

3.1 Kit components and storage

Component	Quantity	Feature	Storage
Plate (Microwell plate coated with SARS-CoV-2 S1 recombinant protein)	12 strips of 8 wells (96 wells)	colorless polystyrene plate	2-8°C
Standard (SARS-CoV-2 S1 antibody)	1ml x 1bottle (8ng)	colorless liquid	2-8°C
Standard Diluent	10ml x 1 bottle	clear yellow liquid	2-8°C
Sample Diluent	100ml x 1 bottle	colorless liquid	2-8°C
Conjugate (100X)	0.15ml x 1 bottle	clear orange liquid	2-8°C
Conjugate Diluent	13ml x 1 bottle	clear orange liquid	2-8°C
Washing Solution, 20X	50ml x 1 bottle	colorless liquid	2-8°C
TMB Substrate (tetramethyl benzidine)	12ml x 1 bottle	colorless liquid	2-8°C
Stop Solution (1N HCL)	12ml x 1 bottle	colorless liquid	2-8°C
Calibrator (SARS-Cov-2-S1 monoclonal antibody)	1ml x 1 bottle	colorless liquid	2-8°C

Table 1. Kit Components and Storage Recommendations

3.2 Materials required but not provided

- Calibrated 20ul, 200ul and 1000ul pipette
- 8-channel multi-pipette (50ul and 100ul)
- Double-distilled water, or equivalent or higher grade
- Microplate washer (optional)
- Microplate reader, set to read at 450nm
- Blood collection tube including sodium heparin

4. Precautions

① For research use only. This product is not intended or approved for human, diagnostics or veterinary use.
② Use only after fully reading and understanding these guidelines.
③ The TMB Substrate contains 3,3',5,5'-tetramethyl benzidine, a suspected carcinogen which can be harmful by ingestion, inhalation and eye or skin contact. Use eye protection, wear gloves, and handle with care.
④ The Stop solution contains 1N Hydrochloric acid (HCl) which can be harmful by ingestion, inhalation, and eye or skin contact. Use eye protection, wear gloves and normal laboratory protective clothing. If the stop solution contacts skin or eyes, rinse generously with water and seek medical attention.
⑤ Conjugate Diluent contains bovine serum albumin that can cause allergic reactions. Avoid skin contact.
⑥ Handle human blood as if potentially infectious (HIV, HBV and HCV). Observe relevant blood handling guidelines.
⑦ Wear eye protection, disposable gloves, and wash hands thoroughly after use.
⑧ Do not use kit if any component shows signs of damage or leakage.
⑨ When opening the lid/cap of any reagent tube/bottle or human samples, or when removing their contents, use GLP procedures to avoid microbial contamination or spraying the surroundings.
⑩ DO NOT mix reagents/components from different kit lots.
⑪ DO NOT use expired components.
⑫ When using equipment such as a plate washer or a plate reader, ensure it has been properly calibrated through a regular maintenance schedule.
⑬ When pipetting samples or reagents, use new disposable tips and regularly calibrated pipettes.
⑭ Discard solid waste, unused reagents and biological samples in accordance with Local, Provincial, and Federal regulations.
⑮ Follow general laboratory safety guidelines.

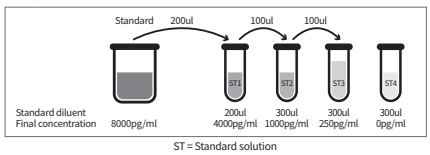
5. Test Procedure

5.1 Preparation Step

- Use plasma samples from blood that has been collected in a blood collection tube coated with sodium heparin (available separately) and prepared as per the product package insert.
- Plasma is separated from the blood after centrifugation.
- Prepare all the standards and samples so that they can promptly be loaded onto the plate. This will avoid significant variations due to the time gap between loading the first and last microwell plate.
- Plasma samples, antibody-coated microwell strips and all reagents must be brought to room temperature just before use.
- Plasma samples must be diluted 1:199 with Sample Diluent. (e.g. 5ul Plasma sample + 995ul Sample Diluent)
- Microwell strips must be brought to room temperature 30 minutes before use. Microwell strips that are not required must be promptly returned to the foil pouch with desiccant, resealed, and returned to the refrigerator for storage until required.
- Washing solution 20X must be diluted 1:19 in advance into purified water (double-distilled or higher grade). Stored at room temperature.

5.2 ELISA Test

5.2.1 Quantitative ELISA Test



ST = Standard solution

Figure 1. Preparation of standard curve dilutions

Standard dilution method (Standard range = 0-4000 pg/mL)			
ST	Standard Diluent(uL)	Standard solution (uL)	Final standard concentration (pg/mL)
1	200	200	4000
2	300	100	1000
3	300	100	250
4	300	0	0=Blank

Table 2. Preparation of standard curve dilutions

② Prepare 2 (duplicate) wells for each of the four standard curve dilutions (4000 to 0 pg/mL), and enough wells for all specimens to be tested. Microwell strips that are not required must be promptly returned to the foil pouch with desiccant, resealed, and returned to the refrigerator for storage until required.
③ Add 100 µL of each dilution of the Standard curve and 100 µL of each sample to wells. Seal plate with the adhesive film provided and lightly tap the frame to ensure proper mixing. Incubate for 1 hour at 37°C. Adhesive film improperly used can cause sample cross-contamination (droplets can adhere to the inner surface). Evaporation can occur due to improper sealing. Use the samples prepared in 5.1 Preparation Step. See Figure 2 below for examples of plate layout:

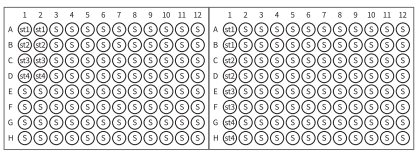


Figure 2. Suggested plate layouts
ST: Standard curve, S: Samples

④ Carefully remove the adhesive film from the plate and aspirate liquid from all wells. Wash 4 times by filling each well with 300 µL of Wash Buffer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash buffer by inverting the plate and tapping vigorously on clean dry paper towels. Before aspirating the wash solution from the 4th cycle make sure Detection Solution has been prepared (see next step) to prevent drying of the wells.
⑤ Prepare Detection Solution by diluting Conjugate 1:99 into Conjugate Diluent.
Example: To prepare 10 mL, (Use 15 mL conical tube) Conjugate Diluent 9.9 mL + Conjugate 0.1 mL and mix by gentle inversion.
⑥ Add 100 µL of Detection Solution (made in step ⑤) to each well. Cover plate with adhesive film. Incubate for 1hour at 37°C.
⑦ Carefully remove the adhesive film from the plate and aspirate liquid from all wells. Wash 4 times by filling each well with 300 µL of Wash Buffer. The complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash buffer by inverting the plate and tapping vigorously on clean dry paper towels. Prevent drying of the wells.
⑧ Add 100 µL of TMB Substrate to each well. Incubate for 30 minutes at room temperature and in the dark.
⑨ Protect the TMB Substrate at all times from strong light or sunlight. For optimal reproducibility, always incubate for exactly 30 minutes at room temperature.
⑩ Add 100 µL of Stop Solution to each well. The color in the well should change from blue to yellow. For optimal reproducibility, Stop Solution should be added to wells in the same order and speed as the TMB Substrate in step ⑧.
⑪ Gently tap the plate to ensure thorough mixing. Carefully wipe clean the bottom of the plate with soft absorbent paper to remove residual humidity or foreign substances, and promptly determine the absorbance of each well using a microplate reader set to 450 nm.
Read within 5 minutes since absorbance will slowly decrease over time after adding the Stop Solution.

5.2.2 Qualitative ELISA Test

① Prepare the positive control by diluting Standard 1:1 into Standard Diluent. See Figure 3 below. Standard Diluent (100uL) is used for the negative control. See Figure 4 below.

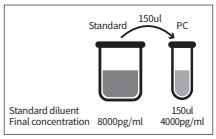


Figure 3. Preparation of positive control

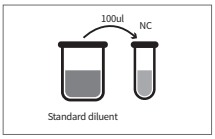


Figure 4. Preparation of negative control

② Prepare microwell strips that are required for samples, positive control, negative control and calibrator. Microwell strips that are not required must be promptly returned to the foil pouch with desiccant, resealed, and returned to the refrigerator for storage until required.
③ Add 100 µL of diluted positive controls, negative controls, and non-diluted -ready-to-use-calibrators to wells, respectively. Seal plate with the adhesive film provided and lightly tap the frame to ensure proper mixing. Incubate for 1 hour at 37°C. Adhesive film improperly used can cause sample cross-contamination (droplets can adhere to the inner surface). Evaporation can occur due to improper sealing. See Figure 5 below for examples of plate layout: Use the samples prepared in 5.1 Preparation Step

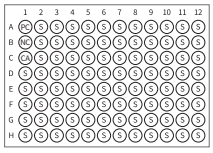


Figure 5. Suggested plate layout
PC: Positive control, NC: Negative control, CA: Calibrators, S: Samples

④ Carefully remove the adhesive film from the plate and aspirate liquid from all wells. Wash 4 times by filling each well with 300 µL of Wash Buffer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash buffer by inverting the plate and tapping vigorously on clean dry paper towels. Before aspirating the wash solution from the 4th cycle make sure Detection Solution has been prepared (see next step) to prevent drying of the wells.
⑤ Prepare Detection Solution by diluting Conjugate 1:99 into Conjugate Diluent.
Example: To prepare 10 mL, (Use 15 mL conical tube) Conjugate Diluent 9.9 mL + Conjugate 0.1 mL and mix by gentle inversion.
⑥ Add 100 µL of Detection Solution (made in step ⑤) to each well. Cover plate with adhesive film. Incubate for 1hour at 37°C.
⑦ Carefully remove the adhesive film from the plate and aspirate liquid from all wells. Wash 4 times by filling each well with 300 µL of Wash Buffer. The complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash buffer by inverting the plate and tapping vigorously on clean dry paper towels. Prevent drying of the wells.
⑧ Add 100 µL of TMB Substrate to each well. Incubate for 30 minutes at room temperature and in the dark.
⑨ Protect the TMB Substrate at all times from strong light or sunlight. For optimal reproducibility, always incubate for exactly 30 minutes at room temperature.
⑩ Add 100 µL of Stop Solution to each well. The color in the well should change from blue to yellow. For optimal reproducibility, Stop Solution should be added to wells in the same order and speed as the TMB Substrate in step ⑧.
⑪ Gently tap the plate to ensure thorough mixing. Carefully wipe clean the bottom of the plate with soft absorbent paper to remove residual humidity or foreign substances, and promptly determine the absorbance of each well using a microplate reader set to 450 nm.
Read within 5 minutes since absorbance will slowly decrease over time after adding the Stop Solution.

6. Calculation and result analysis

6.1 Quantitative Interpretation

6.1.1 Standard curve calculation

Perform linear regression analysis on the standard curve. The X axis shows the concentration of standard solution, in pg/mL, and the Y axis the (corrected) absorbance at 450 nm. The correlation coefficient (R2) of the linear regression of the standard curve must be ≥ 0.98. A numerical example of the standard curve calculation is shown below.

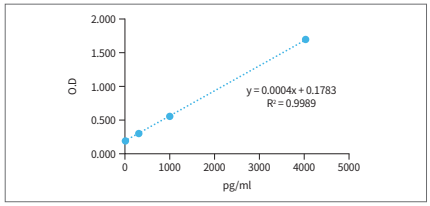


Figure 6. Example of standard curve

6.1.2 Quality Control

The accuracy of test results depends on the generation of an accurate standard curve. Therefore, results derived from the standards must be consistent before test sample results can be interpreted.
For the ELISA to be valid:
① **Raw absorbance value (not blanked) of Standard 1 must be ≥ 1.2**
② **Raw absorbance value (not blanked) of Standard 4 must be ≤ 0.2**
③ **The correlation coefficient (R2) of the linear regression of the standard curve must be ≥ 0.98**
If these conditions are not satisfied, the test results are not valid and a new test must be conducted.

6.1.3 Calculation of Sample Concentration

Calculate the concentration of the samples using the average of the absorbance values and the equation generated by linear regression analysis (y=ax+b). For instance, if the latter is y=0.0004x+0.1783 (as shown in the example), and the sample average absorbance is 0.845, then the sample concentration is calculated as follows:
Sample concentration (pg/mL) = (average absorbance of the sample – b) / a
Sample concentration (pg/mL) = (0.845 – 0.1783) / 0.0004 = 1666.75 (pg/mL)

6.2 Qualitative Interpretation

6.2.1 Interpretation of results

Absorbance of the sample / Absorbance of Calibrator = Ratio

Ratio < 0.9 : Negative

0.9 ≤ Ratio < 1.1 : Borderline

Ratio ≥ 1.1 : Positive

*** If the result is Borderline, re-test after 1~2 weeks because antibodies are created 10~14 days after the infection.**

6.2.2 Quality Control

① **The Ratio value of Negative control must be < 0.9**
② **The Ratio value of Positive control must be ≥ 1.1.**
If these conditions are not satisfied, the test results are not valid, and a new test must be conducted.

7. Limitation

7.1 Results will not be reliable if:

① The test was not properly conducted as per the package insert procedure described.
② The washing step for the plate was not properly conducted as per the package insert.
③ Samples that have an absorbance value falling out of the range of the standard curve (Over 4000pg/ml), These samples should be diluted with the Standard Diluent. When analyzing the results must be multiplied by the dilution factor. (Optional)

7.2 Aggregation of sample

Ensure that plasma samples are completely thawed and centrifuged at 11,500g for 1 min at room temperature immediately before loading them into the ELISA well.

7.3 ELISA Trouble Shooting

Problem	Possible cause	Solution
Non-specific color development / High background	Incomplete/improper washing of the plate	Repeat ELISA, washing with 300 µL of washing solution per well, for the suggested number of times. A soak time of 5 seconds minimum should be used. Eliminate residual wash buffer by inverting plate and vigorously tapping onto absorbent paper.
	Cross-contamination of ELISA well	Pipet samples carefully into microwell strips to minimize risk.
	Expired components/ kit	Ensure kit is used within the expiry date. Diluted standard solution should not be re-used.
	Substrate solution (TMB) is contaminated	Discard if solution appears blue before adding it to the wells.
	Adhesive film improperly used or sealed.	Re-using the adhesive film can cause sample cross-contamination (droplets can adhere to the inner surface). Evaporation can occur due to improper sealing.
Low optical density reading for standard	Standard dilution error	Ensure dilutions of the kit standard are prepared correctly as per the package insert.
	Pipetting error	Calibrate the pipette and conduct correctly as per the package insert.
	Temperature too low	The test is performed at room temperature (22±5°C).
	Incubation time too short	The substrate solution (TMB) must be incubated for exactly 30 minutes.
	Incorrect plate reader filter used	Plate should be read at 450 nm
	Temperature of the solutions too low	Bring solutions to room temperature before use.
	Kit /components have expired	Ensure kit is used within the expiry date.
Non-linear standard curve. High variability of the replicates	Incomplete/improper washing of the plate	Repeat ELISA washing with 300 µL of washing solution per well, for the suggested number of times. A soak time of 5 seconds minimum should be used. Invert plate and tap onto absorbent paper to eliminate residual wash buffer.
	Standard dilution error	Ensure dilutions of the Kit standard are prepared as per the Package insert.
	Poor mixing	Mix reagents thoroughly prior to their use or addition to the plate.

8. Technical Service

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