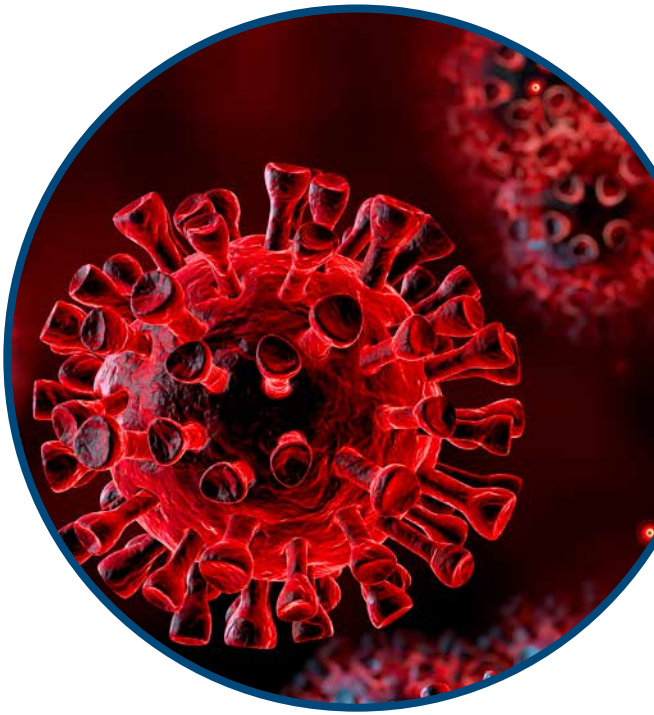
Instruction Manual

Human Anti-SARS-CoV-2 IgG ELISA Kit

For quantitative detection of Anti-SARS-CoV-2 IgG in serum, plasma and other biological fluids from human



Cat. No. 08440100

Size: 96 Tests

Reactivity: Human

Range: 3.906–250 ng/mL

Sensitivity: 2.344 ng/mL

Storage: 2–8°C for 6 months

Principle: Indirect ELISA

Revision Date: 2020-06

For Research Use Only

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Table of Contents

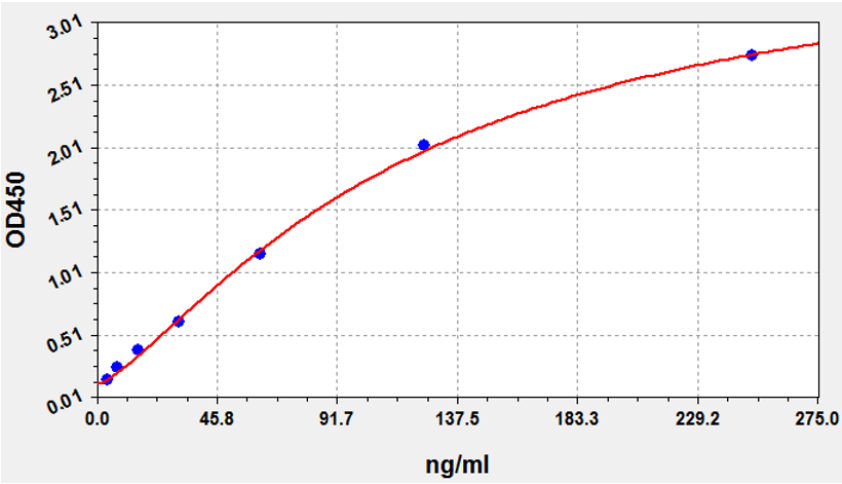
1. Kit Components

No.	Item	Specs (96T)	Storage
E001	ELISA Microplate (Dismountable)	8×12	2–8°C/-20°C
E002	Standard	1 vial	2–8°C
E039	Sample/Standard Dilution Buffer	20 mL	2–8°C
E003	HRP-labeled Antibody (Concentrated)	60 µL	2–8°C (protect from light)
E040	Antibody Dilution Buffer	5 mL	2–8°C
E024	TMB Substrate	5 mL	2–8°C (protect from light)
E026	Stop Solution	5 mL	2–8°C
E038	Wash Buffer (25X)	30 mL	2–8°C
E006	Plate Sealer	5 pieces	
E007	Product Description	1 copy	

2. Typical Data & Standard Curve

Results of a typical standard operation of an Anti-SARS-CoV-2 IgG ELISA Kit are listed below. This standard curve was generated at our lab for demonstration purposes only. Users shall obtain a standard curve via their own experimental results.
(N/A = not applicable)

STD (ng/mL)	OD-1	OD-2	Average	Corrected
0	0.077	0.079	0.078	0.000
3.906	0.145	0.153	0.149	0.071
7.812	0.246	0.258	0.252	0.174
15.625	0.397	0.379	0.388	0.310
31.25	0.610	0.628	0.619	0.541
62.5	1.131	1.189	1.16	1.082
125	2.054	2.004	2.029	1.951
250	2.677	2.811	2.744	2.666



2.1 Sample test results

Samples came from rehabilitation clients of mobile cabin hospital. The plasma samples were diluted 1:100. TMB Color development time was 10 minutes at 37°C .

Due to individual differences, it is recommended to dilute the sample 1:50–1:2000.

Rehabilitation clients (μg/mL)				Healthy volunteers (μg/mL)			
#1	31	#5	35	#1	< 0.1	#5	not detected
#2	7	#6	17	#2	not detected	#6	not detected
#3	24	#7	38	#3	not detected	#7	not detected
#4	47	#8	0.6	#4	not detected	#8	not detected

2.2 Specificity

This assay has high sensitivity and excellent specificity for detection of Anti-SARS-CoV-2 IgG. No significant cross-reactivity or interference between Anti-SARS-CoV-2 IgG and analogues was observed.

NOTE: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between Anti-SARS-CoV-2 IgG and all the analogues; therefore, cross reaction may still exist.

2.3 Recovery

Matrices listed below were spiked with various levels of Anti-SARS-CoV-2 IgG and the recovery rates were calculated by comparing the measured value to the expected amount of Anti-SARS-CoV-2 IgG in samples.

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	87-105	95
EDTA Plasma (n=5)	90-103	97
Heparin Plasma (n=5)	86-104	97

2.4 Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentrations of Anti-SARS-CoV-2 IgG and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8
Serum (n=5)	88-102%	87-105%	91-103%
EDTA Plasma (n=5)	86-101%	83-101%	82-98%
Heparin Plasma (n=5)	81-98%	83-97%	90-96%

2.5 Precision

Intra-Assay: CV < 8%
Inter-Assay: CV < 10%

2.6 Stability

The stability of the ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage conditions.

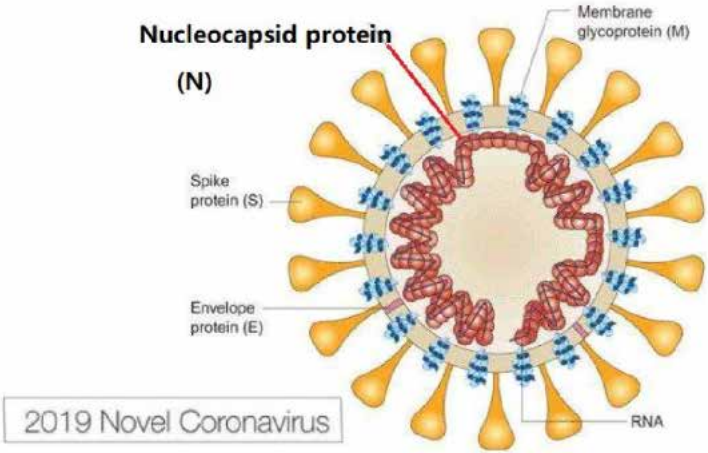
Standard (n=5)	37 °C for 1 month	2–8 °C for 6 months
Average (%)	80	95-100

To minimize extra influence on performance, operation procedures and lab conditions, especially room temperature, air humidity, and incubator temperatures should be strictly controlled. It is strongly suggested that the same operator perform the entire assay from beginning to end.

3. Operation Procedure

3.1 Principle of the Assay

This kit was based on indirect enzyme-linked immunosorbent assay technology. Antigen was pre-coated onto 96-well plates. The HRP-conjugated antibody was used as detection antibodies. The standards, test samples and HRP-conjugated detection antibody were added to the wells subsequently and washed with wash buffer. TMB substrates were used to visualize the HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in the plate. Read the O.D. absorbance at 450 nm in a microplate reader to calculate the concentration of target.



Sequence of Nucleocapsid protein (antigen)

MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTAS
WFTALTQHGKEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPR
WYFYYLGTGPEAGLPYGANKDGIIWVATEGALNTPKDHIGTRNPANNAAIVLQLPQGT
TLPKGFYAEGSRGGSQASSRSSSRSRNSSRNSTPGSSRGTSPARMAGNGGDAA
LALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKAYNVTQA
FGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPS
GTWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKADETQALPQRQK
KQQTVTLLPAADLDDFSKQLQQSMSSADSTQA

3.2 Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion,a pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Store TMB reagents to avoid light.
5. The washing process is very important; incomplete washing can cause a false positive reading and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Avoid letting the microplate dry during the assay - dry plates may inactivate components on the plate.
8. Do not re-use tips and tubes to avoid cross contamination.
9. Avoid mixing reagents from different batches to perform an assay.

3.3 Materials Required, But Not Supplied

- Microplate reader (wavelength: 450 nm)
- 37°C incubator
- Automated plate washer
- Precision single and multi-channel pipette and disposable tips
- Clean tubes and Eppendorf tubes
- Deionized or distilled water

3.4 Washing

Manual: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350 µL Wash Buffer and soak for 1 to 2 minutes, then aspirate contents from the plate and clap the plate on absorbent filter paper or other absorbent material.

Automated: Aspirate all wells, then wash plate with 350 µL Wash Buffer. After the final wash, invert plate and clap the plate on absorbent filter paper or other absorbent material. It is recommended that the washer shall be set to soak for 1 minute.
NOTE: Set the height of the needles; be sure the fluid can be sipped up completely.

3.5 Sample Collection and Storage (universal)

- **Serum:** Place whole blood sample at room temperature for 2 hours or place at 2–8°C overnight, then centrifuge for 20 minutes at approximately 1000 × g. Collect the supernatant and perform the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and free from endotoxins.

- **Plasma:** Collect plasma using EDTA-Na_2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 × g at 2–8°C within 30 minutes of collection. Collect the supernatant and perform the assay immediately. Avoid hemolyzed, high cholesterol samples.

- **Other Biological Fluids:** Centrifuge samples for 20 minutes at 1000 × g at 2–8°C. Collect supernatant and carry out the assay immediately.

NOTE: Samples to be used within 5 days can be stored at 2–8°C; otherwise, store samples at -20°C (assay ≤ 1 month) or -80°C (assay ≤ 2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. Hemolyzed samples are not suitable for this assay.

3.6 Sample Dilution

The user should estimate the concentration of target protein in the test sample and select a proper dilution factor to ensure the diluted target protein concentration falls within the optimal detection range of the kit. Dilute the sample with the provided dilution buffer; several trials may be necessary. The test sample must be well mixed with the dilution buffer and standard curves and sample should be made during the pre-experiment. If samples have very high concentrations, dilute samples with PBS first, then dilute with Sample Dilution.

The matrix components in the sample will affect the test results and need to be diluted at least 1:50 with Sample Dilution Buffer before testing!

3.7 Reagent Preparation and Storage

Bring all reagents and samples to room temperature for 20 minutes before use.

1. Wash Buffer:

If crystals have formed in the concentrate, warm with a 40°C water bath (heating temperature should not exceed 50°C) and mix gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

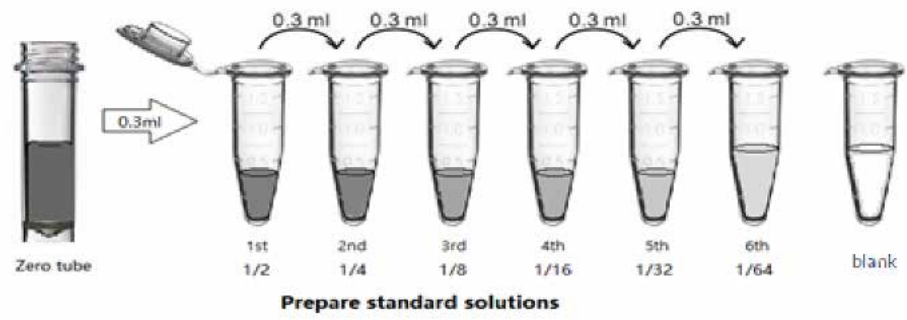
Dilute 30 mL Concentrated Wash Buffer into 750 mL Wash Buffer with deionized or distilled water. Return unused solution back to 2–8°C for storage.

2. Standards:

1. Add 0.3 mL Sample Dilution Buffer into one tube (labeled as zero tube), transfer 0.3 mL from standard tube (500 ng/mL) to zero tube, and mix thoroughly.

NOTE: If the standard tube concentration is higher than the range of the kit, please dilute it and label it as the zero tube.

2. Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank, respectively. Add 0.3 mL of the Sample Dilution Buffer into each tube. Add 0.3 mL of the above Standard Solution (from zero tube) into 1st tube and mix thoroughly. Transfer 0.3 mL from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 mL from 2nd tube to 3rd tube and mix thoroughly, and so on. Sample Dilution Buffer was used for the blank control.



NOTE: It is best to use Standard Solutions within 15 min.

3. Preparation of HRP-labeled Antibody Working Solution:
Prepare within 30 min of initiating the experiment.

1. Calculate required total volume of the working solution: 50 µL / well × quantity of wells. (Allow 55–60 µL more than the total volume.)

2. Dilute the HRP-detection antibody with Antibody Dilution Buffer at 1:100 and mix thoroughly (i.e. add 1 µL HRP-labeled antibody to 99 µL Antibody Dilution Buffer).

3.8 Assay Procedure

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 min at 37°C. It is recommended to plot a standard curve for each test.

1. Set standard, **test samples (diluted at least 1:50 with Sample Dilution Buffer)**, control (blank) wells on the pre-coated plate, respectively, and record their positions. It is recommended to measure each standard and sample in duplicate. **Wash plate 2 times before adding standard, sample and control (blank) wells!**

2. **Prepare Standards:** Aliquot 50 µL of zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube, 5th tube, 6th tube and Sample Dilution Buffer (blank) into the standard wells.

3. **Add Samples:** Add 50 µL of properly diluted sample into test sample wells.

4. **Incubate:** Seal the plate with a cover and incubate at 37°C for 30 minutes.

5. **Wash:** Remove the cover and discard the plate contents, then wash the plate 3 times with Wash Buffer. Do NOT let the wells dry completely at any point.

6. **HRP-labeled Antibody:** Add 50 µL HRP-labeled antibody working solution into above wells (standard, test sample and blank wells). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 30 minutes.

7. **Wash:** Remove the cover and wash plate 5 times with Wash Buffer, then let the Wash Buffer stay in the wells for 1–2 minutes each time.

8. **TMB Substrate:** Add 50 µL TMB Substrate into each well, cover the plate and incubate at 37°C in the dark within 10–15 minutes. (**NOTE:** The reaction time can be decreased or extended according to the actual color change, but not more than 30 minutes. The reaction can be terminated when a gradient appears in standard wells.)

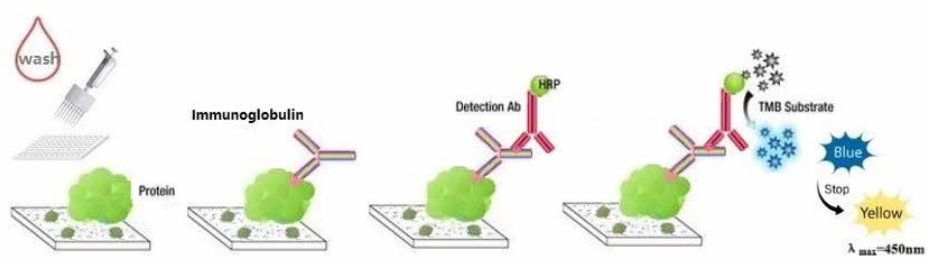
9. **Stop:** Add 50 µL Stop Solution to each well. The color will turn yellow immediately. The addition order of Stop Solution should be the same as the TMB Substrate Solution.

10. **OD Measurement:** Read the O.D. absorbance at 450 nm in a Microplate Reader immediately after adding the Stop Solution.

Regarding calculation, (the relative O.D. 450) = (the O.D. 450 of each well) – (the O.D. 450 of blank well). The standard curve can be plotted as the relative O.D. 450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation, such as Curve Expert 1.3 or 1.4.

NOTE: If the samples measured were diluted, multiply the dilution factor by the concentration from interpolation to obtain the concentration before dilution.

3.9 Summary



Step 1: Wash plate 2 times before adding Standard, **Sample (diluted at least 1:50 with Sample Dilution Buffer)** and Control (blank) wells!

Step 2: Add 50 µL standard or sample to each well and incubate for 30 minutes at 37°C.

Wash step: Aspirate and wash plates 3 times.

Step 3: Add 50 µL HRP-labeled antibody working solution to each well and incubate for 30 minutes at 37°C.

Wash step: Aspirate and wash plates 5 times.

Step 4: Add 50 µL TMB Substrate Solution. Incubate 10–15 minutes at 37°C.

Step 5: Add 50 µL Stop Solution. Read at 450 nm immediately and perform calculations.

4. Related Products

Product Name	Cat. No.
Human anti-SARS-CoV-2 IgM ELISA Kit	08440200
COVID-19 nucleoprotein ELISA Kit, Universal	08440300
Anti-coronavirus (SARS-CoV-2) spike S1, mouse, mAb	0872030
Anti-coronavirus (SARS-CoV-2) spike S2, mouse, mAb	0872040
Anti-coronavirus (SARS-CoV-2) spike S2 (B), mouse, mAb	0872041
SARS-CoV-2 Nucleocapsid Protein, His tag (*E. Coli*)	0872050
ACE2, His tag (*E. Coli*)	0872060

Notes:

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