ACCUCARE®

Novel Coronavirus COVID-19 IgG ELISA Kit
(Serum/Plasma)

INTENDED USE

This kit is intended for the qualitative detection of human anti-COVID- 19 IgG antibody in human serum/plasma.

INDICATIONS FOR USE

This kit is used as an aid for the detection of novel COVID-19. Patients with suspected clustering cases require diagnosis or differential diagnosis of novel coronavirus infection. This kit is for in-vitro diagnostic use only.

SUMMARY OF PHYSIOLOGY

2019 novel coronavirus (COVID-19) is a single-stranded RNA coronavirus. Comparisons of the genetic sequences of this virus have shown similarities to SARS-CoV and batcoronaviruses. In humans, coronaviruses cause respiratory infections. Coronaviruses are composed of several proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N). Results suggest that the spike protein retains sufficient affinity to the Angiotensin converting enzyme 2 (ACE2) receptor to use it as a mechanism of cell entry. Human tohuman transmission of coronaviruses is primarily thought to occur among close contacts via respiratory droplets generated by sneezing and coughing. IgG is the most abundantly found immunoglobulin to beproduced in response to an antigen and will be maintained in the bodyafter initial exposure for long term response.

ASSAY PRINCIPLE

This ELISA kit is designed, developed, and produced for the qualitative measurement of the COVID-19 IgG antibody in serum. This assay utilizes the microplate based enzyme immunoassaytechnique.

Assay controls and 1:10 diluted human serum samples are added to the microtiter wells of a microplate that was coated with COVID-19 recombinant full lengthrecombinant protein. After the first incubationperiod, the unbound protein matrix is removed with a subsequentwashing step. A horseradish peroxidase (HRP) labeled polyclonal goatanti-human IgG tracer antibody is added to each well. After anincubation period, an immunocomplex of "COVID-19 recombinantantigen – human anti-COVID-19 IgG antibody - HRP labeled antihuman IgG tracer antibody" is formed if there is specific Coronavirus IgG antibody present in the tested specimen. The unbound tracerantibody is removed by the subsequent washing step. HRP-Labeled tracer antibody bound to the well is then incubated with a substratesolution in a timed reaction and then measured in a spectrophotometricmicroplate reader. The enzymatic activity of the tracer antibody boundto the anti-COVID-19 IgG on the wall of the microtiter well isproportional to the amount of the anti-COVID-19 IgG antibody level inthe tested specimen.

REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 – 8℃ upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

1. COVID-19 Antigen Coated Microplate
Microplate coated with COVID-19 recombinant antigen.
Qty: 1 x 96 well microplate
Storage: 2 – 8℃
Preparation: **Ready to use.**

2. COVID-19 IgG Sample Diluent
A ready-to-use sample dilution buffer.
Qty: 1 x 15 mL
Storage: 2 – 8℃
Preparation: **Ready to use.**

3. HRP Labeled Anti-hIgG Antibody (100X)
HRP labeled polyclonal goat anti-humanstabilized protein matrix.
Qty: 1 x 0.2 mL (200 µL)
Storage: 2 – 8℃
Preparation: **Refer Preparation section in 4. Conjugate diluent / 3. Conjugate Preparation in Assay Procedure.**

4. Conjugate Diluent
A ready to use Conjugate Diluent
Qty: 1 x 15 mL
Storage: 2 – 8℃
Preparation: Dilution 1:100 (10µL of Conjugate in 990µL of Conjugate Diluent.

5. ELISA Wash Concentrate (20X)
Surfactant in a phosphate buffered saline with non-azidepreservative.
Qty: 1 x 20 mL
Storage: 2 – 25℃
Preparation: 20X Concentrate. The contents must be diluted with 380 mL distilled water and mixed well before use.

6. ELISA TMB Substrate
Tetramethylbenzidine (TMB) with stabilized hydrogenperoxide.
Qty: 1 x 15 mL
Storage: 2 – 8℃
Preparation: **Ready to use.**

7. ELISA Stop Solution
0.5 M sulfuric acid.
Qty: 1 x 10 mL
Storage: 2 – 25℃
Preparation: **Ready to use.**

8. COVID-19 IgG Negative Control
Negative control with a bovine serum albumin based matrixwith non-azide preservative. Control products do not containany serum from patients with new type of Coronavirus infection.
Qty: 1 x 0.6 mL
Storage: 2 – 8℃.
Preparation: **Ready to use.**

9. COVID-19 IgG Positive Control
Positive control with a bovine serum albumin based matrixwith non-azide preservative. Control products do not containany serum from patients with new type of Coronavirus infection.
Qty: 1 x 0.6 mL
Storage: 2 – 8℃.
Preparation: **Ready to use.**

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SAFETY PRECAUTIONS

1. The reagents are for in-vitro diagnostic use only.
2. Wear gloves while performing this assay and handle thespecimen as if they were potentially infectious.
3. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid.
4. Do not get in eyes, on skin, or on clothing.
5. Do not ingest or inhale fumes.
6. On contact, flush with copious amounts of water for at least 15 minutes.
7. Use Good Laboratory Practices.

SAMPLE COLLECTION & STORAGE

Only 20 µL of human serum is required for measurement in duplicate.Samples should only be used on the same day. Severe hemolyticsamples should not be used.

ASSAY PROCEDURE

1. Reagent Preparation

1. Prior to use, allow all reagents to come to room temperature.Reagents from different kit lot numbers should not becombined or interchanged.
2. ELISA Wash Concentrate must be diluted toworking solution prior to use. Please see REAGENTSsection for details.

2. Sample Preparation

1. Dilute sample by a 1:10 dilution ratio with the COVID-19IgG Sample Diluent. For each 10 µL of sample,90µL of COVID-19 IgG Sample Diluent isneeded.
2. Mix well prior to performing the assay.

3. Conjugate Preparation

1. Dilute HRP Labeled Anti-hIgG Antibody (100X) 1:100 dilution with Conjugate Diluent. For example

Conjugate Working Reagent Vol.	HRP Labeled Anti-hIgG Antibody (100X)	Conjugate Diluent
1 mL	10 µL	990 µL
5 mL	50 µL	4950 µL
10 mL	100 µL	9900 µL

3. Assay Procedure

1. Place a sufficient number of microwell stripsin a holder to run controls and samples in duplicate.
2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Blank	SAMPLE 1	SAMPLE 5
B	Blank	SAMPLE 1	SAMPLE 5
C	Negative Control	SAMPLE 2	SAMPLE 6
D	Negative Control	SAMPLE 2	SAMPLE 6
E	Negative Control	SAMPLE 3	SAMPLE 7
F	Positive Control	SAMPLE 3	SAMPLE 7
G	Positive Control	SAMPLE 4	SAMPLE 8
H	Positive Control	SAMPLE4	SAMPLE 8

3. Add 100µL of Sample Diluent into Blank microwells.
4. Add 100µL of controls and 1:10 diluted samples into the designated microwells.
5. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at 37ºC for 30 minutes.
6. Remove the plate sealer. Aspirate the contents of each well. Wash each well 3 times by dispensing 350 µL of diluted wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
7. Add 100 µL of the working reagent of HRP labeled Anti-human IgG Antibody into the microwells.
8. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at 37ºC for 30 minutes.
9. Remove the plate sealer. Aspirate the contents of each well. Wash each well 3 times by dispensing 350 µL of diluted wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
10. Add 100 µL of the substrate into the microwells.
11. Mix gently and cover the plate with aluminum foil. Incubate at room temperature (20-25ºC) for 15 minutes.
12. Remove the aluminum foil and add 50 µL of stop solution into each of the microwells. Mix by gently by tapping the plate.
13. Read the absorbance at primary wavelength 450 nm and secondary wavelength 630nm within 10 minutes with a microplate reader.

Titration Procedure of Positive Specimen

14. Place 10 microtubes for serial dilution
15. Mark tube as Tube 1 to Tube 10 (Dilution 1:10, 1:20, 1:40, 1:80, 1:160, 1:320 1:640, 1:1280, 1:2560 and 1:5120)
16. Add 180µL of Sample diluent into Tube No. 1 and Add 100µL of Sample diluent into Tube No. 2 to 10.
17. Add 20µl Positive Specimen in to Tube No.1 and mix well.
18. Transfer 100µL from Tube No. 1 to Tube No. 2 and Mix well.
19. Transfer 100µL from Tube No. 2 to Tube No. 3 and mix well. Continuous this Serial dilution till Tube No. 10.

	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
SD	180	100	100	100	100	100	100	100	100	100
S	20	100	100	100	100	100	100	100	100	100

*SD: Sample Diluent, S: Specimen, Volume in µL

20. Follow the step 4 to 13 to check the titre value of antibodies in the positive specimen.

PROCEDURAL NOTES

1. It is recommended that all samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original bottles and avoid unnecessary exposure to the light.
3. Store any unused antibody-coated strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.

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6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

QUALITY CONTROL

To assure the validity of the results each assay must include bothnegative and positive controls. The average value of the absorbance ofthe negative control is less than 0.25, and the absorbance of thepositive control is not less than 1.00. We also recommend that allassays include the laboratory's own controls in addition to thoseprovided with this kit.

INTERPRETION OF RESULTS

1. Calculate the average value of the absorbance of the negative control (xNC).
2. Calculate the Assay Cut-off value using the following formulas:

   Cut-off = xNC + 0.25

3. Determine the interpretation of the sample by comparing the OD to the following table:

Interpretation	Interval	Results
Negative	Measured value ≤ xNC + 0.25	The sample does not contain the new coronavirus (COVID-19) IgG related antibody
Positive	Measured value ≥ xNC + 0.25	The sample contains novel coronavirus (COVID-19) IgG antibodies.

Each laboratory should establish its own positive judgment value from the strictlyscreened population. The above positive judgment value is for reference only.

LIMITATIONS OF THE PROCEDURE

1. This test is only for qualitative detection. Test result be the sole basis for clinical diagnosis and treatment. confirmation of infection with novel coronavirus (COVID be combined with the patient's clinical signs in conjunction to other tests.
2. In the first week of the onset of the infection with the novelcoronavirus (COVID-19) patients results may be negative for IgG. In addition, patients with low immunity or other diseases that affect immune function, failure of important systemic organs, and use of drugs that suppress immune function can also lead to negative results of new coronavirus IgG. Previous infection of SARS or other coronavirus strain may cause a light IgG positive in view of similarity of different strains.
3. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
4. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

PERFORMANCE CHARACTERISTICS

Repeatability

The assay control is tested in 10 replicates with a CV of OD valuesless than 10%.

Reproducibility

Three lots were tested with the same samples 10 times with a CV less than 10%.

CLINICAL TESTING

Thenormal healthy patients withsamples collected prior to the COVID-19 outbreak [December3, 2019] (n = 150) and RT-PCR confirmed positive patients in after the second week of the onset of the disease (n = 32). The Positive sample evaluation was done at external Approved Lab. The results are as follows:

	Positive	Negative	Total
Positive	32	2	34
Negative	0	148	148
Total	32	150	182

The diagnostic sensitivity is 100% (>98%)
The diagnostic specificity is 98.67% (>97%)

REFERENCES

1. CDC (2020). Transmission of Novel Coronavirus (COVID-19).
2. Fehr, A. R., & Perlman, S. (2015). Coronaviruses: An Overview of Their Replication and Pathogenesis. Coronaviruses Methods in Molecular Biology, 1-23. Doi: 10.1007/978-1-4939-2438-7_1

GLOSSARY OF SYMBOL

	Consult Instruction for Use	LOT	Lot Number
REF	Catalog Number		Date of Manufacturing
	Store between		Use By or Expiration Date
	Manufacturer	IVD	For *in vitro* Diagnostic use only
	Keep away from sunlight	CONT	Content of the kit
	Tests per Kit		Do Not Use if Damaged
	Do not reuse		Keep Dry

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