TRUSTwell®

COVID-19 IgG ELISA Kit

REF

AE0612

IVD

- 96-well ELISA kit for the qualitative detection of COVID-19 IgG antibodies in human Serum or Plasma
- Store at 2-8°C upon receipt

INTENDED USE

The TRUSTwell COVID-19 IgG ELISA Kit is a solid phase enzyme linked immunosorbent assay for the qualitative detection of COVID-19 IgG antibodies in human serum or plasma. It is intended for professional use only an aid in the diagnosis of infection with SARS-CoV-2. Any reactive specimen with the TRUSTwell COVID-19 IgG ELISA Kit must be confirmed with alternative testing method(s) and clinical findings.

INTRODUCTION

Novel coronavirus is a single-standard RNA coronavirus[1]. Comparisons of the genetic sequences of this virus shown similarities to SARS-CoV and bat coronaviruses[2]. In humans, coronaviruses cause respiratory infections[3]. For virion assembly and infection of CoVs mainly Spike (S), Nucleocapsid (N), Membrane (M), Envelope (E) proteins[4]. 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. IgG is the most abundantly found immunoglobulin to be produced in response to an antigen and will be maintained in the body after initial exposure for term response[5]. The TRUSTwell COVID-19 IgG ELISA Kit is intended for *in vitro* qualitative detection of Anti COVID-19 IgG antibodies in human serum or plasma collected from blood samples suspected for COVID-19 infection. The test is highly sensitive and specific.

TEST PRINCIPLE

The TRUSTwell COVID-19 IgG ELISA Kit is a solid phase enzyme linked immunosorbent assay based on the principle of the indirect EIA technique for the detection of Anti COVID-19 IgG antibodies in human serum or plasma.

1) Solid microwells pre-coated with recombinant SARS-CoV-2 antigens.
2) Liquid conjugates composed of anti-human IgG conjugated with horse radish peroxidase (HRP-anti Human IgG conjugates).

During the assay, IgG antibodies from specimen will bind to the SARS-CoV-2 antigen coated on to the microtiter plate. After the first incubation period, the unbound specimen is then removed by a wash step. In the next step, anti-human IgG HRP binds to captured human IgG antibodies. Unbound conjugate is then removed by washing step. Subsequently, chromogenic substrate is added to the microwells, and the presence of the conjugate complex is shown by a blue color resulting from the reaction between the enzyme and substrate. The reaction is stopped with stop solution and absorbances are read using a spectrophotometer at 450/620-690nm.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	Microwells coated with recombinant COVID-19 antigens	8 wells x 12 strips	AE0612 W
2	COVID-19 IgG Negative Control	0.5 mL	AE0612N
3	COVID-19 IgG Positive Control	0.5 mL	AE0612P
4	Sample Diluent	12 mL	AE0612SD
5	HRP-anti human IgG Conjugate	12 mL	AE0612H
6	Wash Buffer (30 X concentrate)	20 mL	AWE3000
7	TMB Substrate A	6 mL	ATME2000A
8	TMB Substrate B	6 mL	ATME2000B
9	Stop Solution	12 mL	ASE1000
10	ELISA Working Sheet	2 Nos	AE0001ES
11	Product Insert	1 No	PI-AE0612
12	Sealant	3 Nos	N/A

Materials and reagents required but not provided in the kit

1. Pipette capable of delivering 10 µL, 50 µL, and 100 µL volumes with a precision better than 1.5%.
2. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable.
3. Absorbent paper for blotting the microplate wells.
4. Timer.
5. Distilled or de-ionized water.
6. Incubator

STORAGE AND STABILITY

All reagents except the concentrated wash buffer are ready to use as supplied. Store all components at 2-8°C. Do not freeze. Avoid strong light. Ensure that the reagents are brought to room temperature before opening. After removing the desired number of wells, place unused wells in the resealable foil pouch provided with desiccant and return to 2-8°C. All reagents are stable upto the expiration date. Once opened, the kit is stable for 8 weeks at 2-8°C.

WARNING AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Inactivate the samples at 60°C for 30 minutes before use.
3. Bring all reagents to room temperature (18°C-28°C) before use.
4. Do not use the components in any other type of test kit as a substitute for the components in this kit. Do not use expired devices
5. Do not use hemolyzed blood specimen for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mucose. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens. Dispose of all specimens and materials used to perform the test as biohazardous waste.
9. In the beginning of each incubation and after adding Stopping Solution, gently rocking the microwells to ensure thorough mixing. Avoid the formation of air bubbles as which results in inaccurate absorbance values. Avoid splash liquid while rocking or shaking the wells
10. Don't allow the microplate to dry between the end of the washing operation and the reagent distribution.
11. The enzyme reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or substrate B solution.
12. The substrate B solution must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced.
13. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate B.
14. The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.
15. Avoid strong light during color development.

MATERIALS AND REAGENTS

1. Serum specimens should be prepared from whole blood obtained by acceptable venipuncture technique. The kit is designed for use with serum or plasma specimen without additives only.
2. If not tested immediately, the specimens can be stored at 2-8°C for up to 7 days. The specimens should be frozen at -20°C for longer storage. Avoid multiple freeze-thaw cycles. If a specimen is to be shipped, pack in compliance with federal regulations covering the transportation of etiologic agents.
3. Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation before testing.
4. Do not use specimens demonstrating gross lipemia, gross hemolysis or turbidity.
5. Specimens containing sodium azide may interfere with test results.

SPECIMEN COLLECTION AND PREPARATION

1. Bring all reagents to room temperature (22-28°C).
2. Dilute concentrated Wash Buffer 30-fold with water as following:

Plate	DI water	30 X wash buffer	Final volume
Full plate	580 mL	20 mL	600 mL
Half plate	290 mL	10 mL	300 mL
A quarter plate	145 mL	5 mL	150 mL

Warm up the concentrated Wash Buffer at 37˚C to dissolve the precipitant if it appears.

3. Mix each reagent before adding to the test wells.
4. Determine the number of microwells needed and mark on the ELISA Working Sheet with the appropriate information. Positive and Negative Controls require to be run in duplicate to ensure accuracy.

ASSAY PROCEDURE

1. Remove the desired number of strips and secure them in the microwell frame. Reseal un-used strips.
2. Add specimens according to the designation on the ELISA Working Sheet
   - 2.1. Blank wells: Leave the blank well alone (2 wells). Don't add any reagents.
   - 2.2 Control and Test wells: Add 100 µL of Sample Diluent to all the wells (except Blank), then transfer 5 µL of each Positive control (2 wells), Negative Control (2 wells) and test specimen to each test well, respectively.

To ensure better precision, use pipette to handle solution.

3. Gently rock the plate wells for twenty seconds, then cover the plate with sealant.
4. Incubate the wells at 37°C for 30 minutes
5. Carefully remove the incubation mixture by emptying the solution into a waste container. Fill each well with diluted wash buffer (350 µL per well) and shake gently for 20-30 seconds. Discard the wash solution completely and tapping the plate on absorbent paper. Repeat above procedure 4 more times.

6. Add 100 µL of HRP- anti-human IgG conjugates into each well except the blank well, cover the plate,
7. Incubate at 37°C for 30 minutes.
8. Wash the plate 5 times as step 5 described.
9. Add 50 µL of TMB substrate A and Add 50 µL of TMB substrate B into each well including the blank well.
10. Incubate at 37°C in dark for 20 minutes.
11. Stop the reaction by adding 100 µL of stop solution to each well. Gently mix for 20 seconds. It is important to make sure that all the blue color changes to yellow color completely.
12. Set the microplate reader wavelength at 450nm and measure the absorbance (OD) of each well against the blank well within 10 minutes after adding Stop Solution. A filter of 620-690nm can be used as a reference Wavelength to optimize the assay result.

Flow chart of assay procedure

1.	Secure strips in microwell frame		Number of strips
2.	Add sample diluent		100 µL
3.	Add controls or specimens Gently rock		5 µL 10 seconds
4.	Incubate		37°C, 30 minutes
5.	Wash: manual or automatic		5 times
6.	Add Conjugate Gently rock		100 µL 20 seconds
7.	Incubate		37°C, 30 minutes
8.	Wash: manual or automatic		5 times
9.	Add TMB A&B substrate Gently rock		50+50 µL 20 seconds
10.	Incubate in dark		37°C, 20 minutes
11.	Add Stop Solution. Gently mix		100 µL 20 seconds
12.	Read the result		450/620-690 nm within 10 minutes

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cut-off value = 0.2 + NC

NC: Mean OD of the negative control. Use 0.05 for calculation of the cut- off value if less than 0.05.

B. Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the Cut-off Value as follows:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cut -off Value}}$$

C. Assay validation

The mean OD value of the COVID-19 IgG positive controls should be ≥ 0.50.
The mean OD value of the COVID-19 IgG negative controls should be ≤ 0.10.

If above specification is not met, the assay is Invalid. Check the assay procedure including incubation time and temperature and repeat assay.

D. Interpretation of the results

	Specimen OD ratio
Negative	< 1.00
Positive	≥ 1.00

1. The negative result indicates that there is no COVID-19 IgG in the specimen.
2. Results just below the cut-off value (Lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to retest in duplicate the corresponding specimens when it is applicable).
3. Specimens with cut-off ≥ 1.00 are initially considered to be positive by the TRUSTwell COVID-19 IgG ELISA Kit. They should be retested in duplicate before final interpretation.

If after re-testing of a specimen, the absorbance value of the 2 duplicates are less than the cut-off value, the initial result is non repeatable and the specimen is considered to be negative with the TRUSTwell COVID-19 IgG ELISA Kit.
Non repeatable reactions are often caused by:

- Inadequate microwell washing,
- Contamination of negative specimens by serum or plasma with a high antibody titer,
- Contamination of the substrate solution by oxidizing agents (bleach,metal ions, etc.)
- Contamination of the stopping solution

If after retesting the absorbance of one of the duplicates is equal or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the TRUSTwell COVID-19 IgG ELISA Kit, subject to the limitation of the procedure, described below.

PERFORMANCE CHARACTERISTICS

Clinical Performance

A total of 707 patient specimens from susceptible subjects were tested by the TRUSTwell COVID-19 IgG ELISA Kit. Comparison for all subjects is showed in the following table:

	TRUSTwell COVID-19 IgG ELISA Kit		
Ref. EIA	**Positive**	**Negative**	**Total**
Positive	150	0	150
Negative	1	556	557
Total	151	556	707

Relative Sensitivity:100%, Relative Specificity:99.82%, Overall Agreement:99.86%

Precision

Intra-assay precision was determined by assaying 20 replicates of three negatives, three weak positives and three strong positive

Specimens	Number of specimens	No. of replicates	CV
Negatives	3	20	5.0 - 10.0 %
High positives	3	20	2.0 - 5.0 %
Low positives	3	20	3.0 – 5.0 %

Cross Reactivity

No false positive COVID-19 IgG ELISA test results were observed on 5 positives specimens from each of the following disease states or special conditions, respectively:

Influenza A & B Dengue HIV HBsAg HCV

LIMITATION OF THE TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of COVID-19 IgG in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The TRUSTwell COVID-19 IgG ELISA Kit is limited to the qualitative detection of COVID-19 IgG in human serum or plasma. The intensity of the color does not have linear correlation with the antibody titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable COVID-19 IgG. However, a negative test result does not preclude the possibility of exposure to or infection with COVID-19.
4. A negative result can occur if the quantity of COVID-19 IgG present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a specimen is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. Chenjia Yuan, Shi Jinsong , Qiudong An , Liu Chang , Li Xin , Qiang , Ruanji Shou , mountains . Wuhan 2019 Bioinformatics coronavirus genome analysis [J / OL]. Bioinformatics: 1-10 [2020-02-10].
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.
3. Li, F., Li, W., Farzan, M., & Harrison, S. (2005). Structure of SARS coronavirus spike receptor-binding domain complexed with its receptor. doi: 10.2210/pdb2ajf/pdb.
4. Xu, X., Chen, P., Wang, J., Feng, J., Zhou, H., Li, X., ... Hao, P. (2020). Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Science China Life Sciences. doi: 10.1007/s11427-020-1637-5.
5. Wu, L.-P., Wang, N.-C., Chang, Y.-H., Tian, X.-Y., Na, D.-Y., Zhang, L.-Y., ... Liang, G.-D. (2007). Duration of Antibody Responses after Severe Acute Respiratory Syndrome. Emerging Infectious Diseases, 13(10), 1562–1564. doi: 10.3201/eid1310.070576.

Index of Symbols

Consult instructions for use	REF Catalogue number	Caution
IVD *In vitro* diagnostic medical device	LOT Batch code	Non-sterile
2°C 8°C Temperature limit 2-8 °C	Do not re-use	Use-by date
Manufacturer	Date of manufacture	Keep away from sunlight
Do not use if package is damaged	Σ Contains sufficient for <n> tests	

Athenese-Dx Pvt. Ltd.
Module No. 407 & 408, 4th Floor,
TICEL Bio Park II, No. 5, CSIR Road.
Taramani, Chennai-600113, India
Tel: +91-44-22541131
E-mail: info@athenesedx.com
Website: www.athenesedx.com

PI-AE0612 Rev. D
Effective date: 02.05.2023
English version