Human SARS-CoV-2 IgG antibody ELISA Kit

Enzyme Immunoassay for the quantification of Human's SARS-CoV-2 IgG antibody in serum (venous, capillary) and plasma (EDTA, heparin, citrate) (1:101 diluted).

Catalog number: ARG82809

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus that causes COVID-19 (coronavirus disease 2019), the respiratory illness responsible for the COVID-19 pandemic. Colloquially known as simply the coronavirus, it was previously referred to by its provisional name, 2019 novel coronavirus (2019-nCoV), and has also been called human coronavirus 2019 (HCoV-19 or hCoV-19). The World Health Organization declared the outbreak a Public Health Emergency of International Concern on 30 January 2020, and a pandemic on 11 March 2020.

SARS-CoV-2 is a positive-sense single-stranded RNA virus (and hence Baltimore class IV) that is contagious in humans. As described by the US National Institutes of Health, it is the successor to SARS-CoV-1, the virus that caused the 2002–2004 SARS outbreak.

Taxonomically, SARS-CoV-2 is a virus of the species severe acute respiratory syndrome–related coronavirus (SARSr-CoV). It is believed to have zoonotic origins and has close genetic similarity to bat coronaviruses, suggesting it emerged from a bat-borne virus. Research is ongoing as to whether SARS-CoV-2 came directly from bats or indirectly through any intermediate hosts. The virus shows little genetic diversity, indicating that the spillover event introducing SARS-CoV-2 to humans is likely to have occurred in late 2019. [Provide by Wikipedia: Severe acute respiratory syndrome coronavirus 2]

PRINCIPLE OF THE ASSAY

This assay employs the enzyme-linked immunosorbent technique. The specific antigens (SARS-CoV-2 Spike antigens) has been pre-coated onto a microtiter plate. The diluted samples, Standards or Controls are added to the pre-coated ELISA plate. After incubation, the wells are washed with diluted Wash Buffer to remove unbound material. Then Antibody Conjugate is added and incubated. After incubation, the wells are washed with diluent Wash Buffer to remove unbound material. Then the TMB substrate is added to the wells and color develops in proportion to the amount of specific antibody binding in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. And run validation to get qualitative result by Blank, Negative Control and Positive Control or quantitative result by Standards and calculate the Units (U) of samples.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Protein Binding microplate	8 X 12 strips	4°C
Standards 1-5 (209, 104, 52, 26, 13 AU/mL)	5 vial, 2 mL each (ready to use)	
Control 1 & 2	2 mL (ready to use)	4°C
Diluent Buffer	50 mL (ready to use)	4°C
Antibody Conjugate (Anti-Human IgG conjugate HRP)	13.5 mL (ready to use)	4°C (protect from light)
20X Wash Buffer	50 mL	4°C
TMB substrate	13.5 mL (ready to use)	4°C (protect from light)
STOP solution	7.5 mL (ready to use)	4°C
Cover foil	3 ea	4°C

Note: QC certificate with information about concentrations and target values of Standards 1-5 and Control 1 & 2.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Incubator (37°C)
- Votex mixer
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- The remaining strips should be immediately resealed in the aluminum foil along with the desiccant supplied and stored at 2-8 °C.
- Briefly spin down the all vials before use.
- It is important to bring all reagents and samples to room temperature (20-25°C) and mix them before starting the test run.
- If crystals are observed in the 20X Wash buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.

- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, do NOT use it.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Use a new adhesive plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Collect the plasma layer and store on ice.

Sample Dilution: Before assaying, all samples should be diluted 1+100 with Diluent Buffer. Dispense 10 µL sample and 1 mL Diluent Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a vortex mixer.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Do not use heat inactivation specimen.
3. Do not use whole blood directly with the assay.
4. Avoid disturbing the white buffy layer when collection serum / plasma sample.
5. Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying. Aliquot samples for testing and store at -80°C. Avoid repeated freeze-thaw cycles. Perform dilutions in Diluent buffer as necessary.
6. Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 20X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 25 mL of 20X Wash Buffer into 475 mL of distilled water to a final volume of 500 mL) The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use.

For quantitative assay procedure, Standards 1-5 (in duplicate), Diluent Buffer, Controls 1 & 2 must be pipetted.

1. Add 100 µL of the diluted samples, Standards (in duplicate) and Controls to each well, and leave a well as blank (100 µL of the Diluent Buffer).
2. Cover wells with the foil and incubate at 37°C for 30 minutes on a microplate shaker.
3. Remove the foil, aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with 1× Wash Buffer (300-350 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. The interval between washing and aspiration should be > 5 sec. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 µL of Antibody Conjugate to each well.
5. Cover wells with the foil and incubate at 37°C for 30 minutes on a

microplate shaker. Do not expose to direct sunlight.

6. Aspirate each well and **wash as step 3**.
7. Add **100 µL** of **TMB Substrate** to each well.
8. Cover wells with the foil and incubate at **37°C** for **30 minutes** in the dark.
9. Add **50 µL** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at **450nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance **within 60 minutes** after adding the stop solution.

For qualitative assay procedure, the Diluent Buffer, Standard 4 (in duplicate), Controls 1 &2 must be pipetted.

1. Add **100 µL** of the **diluted samples, Standard 4 (in duplicate)** and **Controls** to each well, and leave a well as blank (**100 µL** of the **Diluent Buffer**).
2. Cover wells with the foil and incubate at **37°C** for **30 minutes** on a microplate shaker.
3. Remove the foil, aspirate each well and wash, repeating the process 3 times for a total **4 washes.** Wash by filling each well with **1× Wash Buffer (300-350 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. The interval between washing and aspiration should be > 5 sec. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of **Antibody Conjugate** to each well.
5. Cover wells with the foil and incubate at **37°C** for **30 minutes** on a

microplate shaker. Do not expose to direct sunlight.

6. Aspirate each well and **wash as step 3**.
7. Add **100 µL** of **TMB Substrate** to each well.
8. Cover wells with the foil and incubate at **37°C** for **30 minutes** in the dark.
9. Add **50 µL** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at **450nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance **within 60 minutes** after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for Blank, Controls, Standards and diluted samples.
2. The OD values of the blank, Control 1 & 2, and Standard 1-5 must meet the specifications stated in the QC certificate. The value of the calculated units of the Control 1 & 2 must be within the range specified in the QC certificate. If any of the above requirements for OD values or unit values are not met, the test must be repeated.
3. Qualitative Result:

   Calculation of Cut-off

   Correction Factor (CF): To differentiate between positive and negative samples a Cut-off value has been defined. This Cut-off value is correlated with the specific Standard by a lot-specific Correction Factor (refer to QC certificate).

   The Cut-off OD value is the mean absorbance value of the Standard 4 determinations x Correction Factor (CF):

   OD Cut-off (10 AU) = (Standard OD1 + Standard OD2) / 2 X CF

   Results in Arbitrary Unit

   (Sample (mean) absorbance value X 10) / Cut-off = AU (Arbitrary Units)

   E.g. OD_{sample} = 1.591; $OD_{Cut\text{-}off}$ = 0.43, (1.591 X 10) / 0.43 = 37 AU
4. Quantitative Result:

   In order to obtain quantitative results in AU/mL or IU/mL plot the (mean) absorbance values of Standards 1–5 and the Blank on the y-axis against their corresponding concentrations (refer to the QC certificate) on the x-axis and draw the best-fit curve through the plotted points. Read results

from this Standard curve employing the (mean) absorbance values of each sample and control.

5. Interpretation of Results:

Qualitative	Quantitative	Interpretation
<9AU	<9AU/mL	No significant level of antibodies to SARS-CoV-2 in patient sample. Result does however not exclude infection with SARS-CoV-2. Serological evidence is best obtained by testing of paired acute and convalescentphase samples obtained several weeks apart.
9-11AU	9-11AU/mL	Equivocal results should only be interpreted as initial evidence for detection of antibodies to SARS-CoV-2. Additional testing is recommended for equivocal test results. Serological evidence is best obtained by testing of paired acute- and convalescent-phase samples obtained 2 to 4 weeks apart. If the result is equivocal again the sample is reported as negative.
>11AU	>11AU/mL	Antibodies to SARS-CoV-2 presumptively detected in patient sample. Contact with the antigen (pathogen resp. vaccine) can be assumed.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

QUALITY ASSURANCE

Intra-assay and Inter-assay precision

The CV value of intra-assay was 3.78-6.37% and inter-assay was 5.52-14.11%.

Diagnostic specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 99.49% (95% confidence interval: 98.16%- 99.94%).

Diagnostic Sensitivity

Days post symptom onset	Number of samples (n)	Positive	Equivocal	Negative	Sensitivity (Eqv excluded)
0-7	12	2	0	10	16.67%
8-14	20	15	1	4	78.95%
≥ 15	34	33	1	0	100%
asymptomatic	80	73	4	3	96.05%

Only with increasing duration of infection the antibody production starts to rise to a detectable level. Individually, this can vary from a few days up to 2 weeks. At the beginning of an infection a negative test result is therefore not a criterion for exclusion of an acute SARS-CoV-2 infection.

Limit of Blank (LoB), Limit of Detection (LoD), Limit of Quantitation (LoQ)

LoB: 2.4 AU/mL

LoD: 3.6 AU/mL

LoQ: 12.3 AU/mL

QC CERTIFICATE

Quantitative Data analysis

Standards	Conc. (AU/mL)	O.D.	O.D. Validation Criteria
Standard 1	209	1.814	>1.0 and > Standard 2
Standard 2	104	1.442	> Standard 3
Standard 3	52	0.983	> Standard 4
Standard 4	26	0.557	> Standard 5
Standard 5	13	0.290	> Blank

Control	O.D.	Value found (AU/mL)	Target Range (AU/mL)
Control 1	1.182	69	45.94-121.10
Control 2	0.007	0.20	0-10
			O.D. Blank < 0.15

Qualitative data analysis

	Correction Factor (CF)
Standard 4	0.470

	O.D.	Target Value AU	Target Range AU
O.D. cut-off	0.26	10	-
Control 1	1.182	45.5	27.29-71.96
Control 2	0.007	0.26	0-5
			O.D. Blank < 0.15