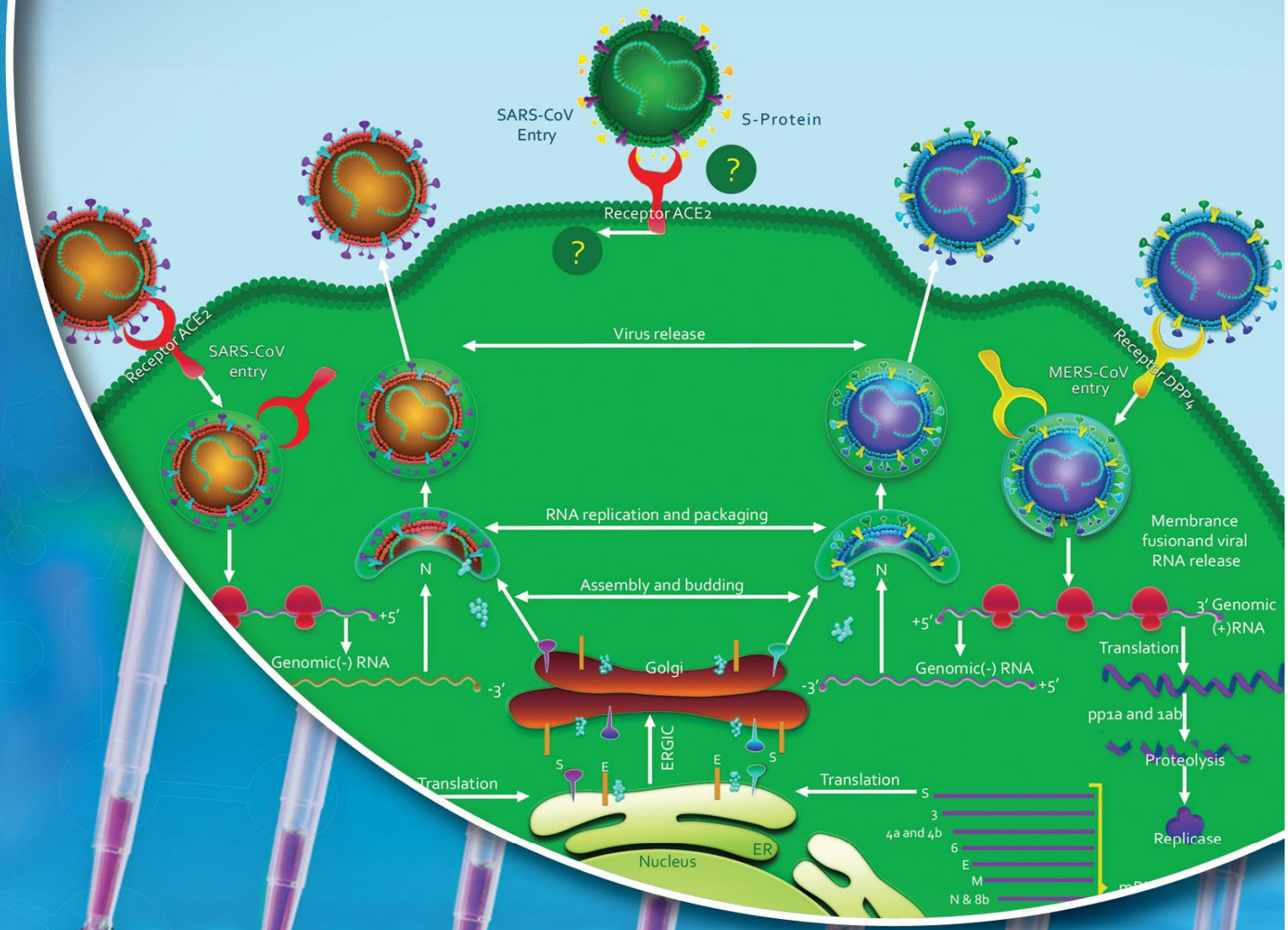
SARS-CoV-2 (Spike RBD) IgG Serological ELISA Kit with False Positive Control



Cat. No. 90-7004

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Antibodies & Engineered Cell Lines™

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1. Intended Use

The SARS-CoV-2 (Spike RBD) IgG Serological ELISA Kit is a sensitive and specific enzyme-linked immunosorbent assay (ELISA) to be used for the qualitative measurement of human Immunoglobulin G (IgG) against SARS-CoV-2 Spike (Receptor Binding Domain) protein in serum and plasma.

2. Introduction

Coronaviruses (CoVs) are enveloped non-segmented positive-sense RNA virus for human and vertebrates. They are classified into four types, α-CoV, β-CoV, γ-CoV, and δ-CoV. They can infect respiratory, gastrointestinal, hepatic, and central nervous system of human and many other wild animals. The family Coronaviridae constantly circulate in the human population and mainly cause mild respiratory diseases. In December 2019, a new severe acute respiratory syndrome β-coronavirus called SARS-CoV-2 (or 2019-nCoV) has emerged, which causes an epidemic of acute respiratory syndrome (called coronavirus human disease 2019 or COVID-19) (1). Typical clinical symptoms of these patients are dry cough, fever, breathing difficulties, headache and pneumonia. Disease onset may result in progressive respiratory failure and even death. SARS-CoV-2 has similarity to SARS-CoV (2).

SARS-CoV-2 virus contains 4 structural proteins, spike (S), envelope (E), membrane (M) and nucleocapsid. The spike protein (S) is a transmembrane protein, composed of the S1 subunit and S2 subunit. The S1 subunit contains a receptor binding domain (RBD), which binds to the cell surface receptor Angiotensin-Converting Enzyme 2 (ACE2) present at the surface of epithelial cells, causing infection of human respiratory cells (3). During an infection, IgM antibody appears first, followed by IgA on mucosal surfaces or IgG in the serum. The spike (S), mainly the RBD domain and the nucleocapsid (N) are the main immunogens of Coronavirus leading to antibody answer (4, 5).

3. General References

(1) A pneumonia outbreak associated with a new coronavirus of probable bat origin: P. Zhou, et al.; Nature 579, 270 (2020)

(2) Identification of a novel coronavirus in patients with severe acute respiratory syndrome: C. Drosten, et al.; N. Engl. J. Med. 348, 1967 (2003)

(3) SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor: M. Hoffmann, et al.; Cell 181, 1 (2020)

(4) Serological assays for emerging coronaviruses: challenges and pitfalls: B. Meyer, et al.; Virus Res. 194, 175 (2014)

(5) Chronological evolution of IgM, IgA, IgG and neutralisation antibodies after infection with SARS-associated coronavirus: P.R. Hsueh, et al., Clin. Microbiol. Infect. 10, 1062 (2004)

4. Assay Principle

This assay is an indirect ELISA assay for qualitative measurement of human anti-SARS-CoV-2 IgG in serum and plasma. Recombinant Spike (RBD) protein of SARS-CoV-2 has been precoated onto the 96-well microtiter plate (Spike Plate). The test samples containing the antibodies against SARS-CoV-2 Spike are pipetted into the wells for binding to the coated viral protein. After extensive washing to remove unbound components, anti-SARS-CoV-2 Spike (RBD) human IgG are detected by the addition of an HRP-conjugated anti-IgG (human) (HRP). Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the levels of anti-SARS-CoV-2 Spike (RBD) human IgG present in the samples. In this assay a second plate (antigen non-coated Background Plate) is provided to determine the sample specific background and to measure the amount of IgG antibodies non-specifically bound to the well. To measure presence of anti-SARS-CoV-2 human IgG antibodies in serum or plasma, net sample optical densities (Net OD) are calculated by subtracting each sample Background Plate OD from the Spike (SARS-CoV-2) antigen plate (Spike Plate) OD.

Note: Positive samples can be further confirmed using our SARS-CoV-2 Antibody "Neutralization" Assay Kit (Cat: # 90-7005).

5. Handling and Storage

- Reagent must be stored at 2-8°C when not in use.
- The validity period is 12 months.
- Plate and reagents should reach room temperature before use.
- Do not expose reagents to temperatures greater than 25°C.

6. Kit Components

• 1 vial anti-human IgG-HRP (100X)	(220 µl)	(HRP 100X)
• 1 vial negative Control (lyophilized)	(for 2 x 2 wells)	(Negative)
• 1 vial positive Control (lyophilized)	(for 2 x 2 wells)	(Positive)
• 2 bottles Wash Buffer 10X	(2 x 30 ml)	(Wash Buffer 10X)
• 1 bottle Sample Buffer 5X	(1 x 30 ml)	(Sample Buffer 5X)
• 1 bottle TMB Substrate Solution	(24 ml)	(TMB)
• 1 bottle Stop Solution	(24 ml)	(STOP)
• 1 plate coated with Spike (RBD)(SARS-CoV-2)	(6 x 16-well strips-Spike)	
• 1 plate Background Control	(6 x 16-well strips-Background)	
• 4 plate Covers (plastic film)		
• 4 silica Gel Minibags.		

7. Materials Required *(but Not Supplied)*

- Microtiterplate reader at 450nm
- Calibrated precision pipettes. Disposable pipette tips
- Deionized water
- Microtubes or equivalent for preparing dilutions
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard
- Black and red permanent markers

8. General ELISA Protocol

8.1. Reagents Preparation and Storage

NOTE: Prepare just the appropriate amount of buffers necessary for the assay.

- Wash Buffer 10X has to be diluted with deionized water 1:10 before use (e.g. 30 ml Wash Buffer 10X + 270 ml water) to obtain Wash Buffer 1X.

- Sample Buffer 5X has to be diluted with deionized water 1:5 before use (e.g. 10 ml Sample Buffer 10X + 40 ml water) to obtain Sample Buffer 1X.

  NOTE: The Sample Buffer 1X is used fresh.

- HRP 100X (HRP Conjugated anti-human IgG) has to be diluted to the working concentration by adding 200 µl in 20 ml of Sample Buffer 1X (1:100) to obtain HRP 1X

  NOTE: The diluted HRP 1X is used fresh.

- Negative Control is reconstituted in 480 µl of deionized water. It should be tested in duplicate in each Background and Spike plate. It contains diluted human serum screened for viral markers.

- Positive Control is reconstituted in 480 µl of deionized water. It should be tested in duplicate in each Background and Spike plate. It contains anti-Spike (RBD) (SARS-CoV-2) monoclonal antibody.

8.2. Sample Collection, Storage and Dilution

Serum: Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at ≤ -20°C for later use. Avoid repeated freeze/thaw cycles.

Plasma: Collect plasma using heparin, citrate or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/ thaw cycles.

Serum and Plasma have to be diluted in Sample Buffer 1X. Each serum/plasma sample should be tested at 1/100 as a start by mixing 5µl of serum / plasma in 495 µl of Sample Buffer 1X. Dilution can be adjusted to the titer of the antibodies in the samples.

NOTES:

- Diluted Serum or Plasma in Sample Buffer 1X should be used fresh.
- Heat inactivation at 56°C for 30 minutes is optional.
- Vortex serum or plasma samples at room temperature to ensure homogeneity. Then centrifuge samples at 10,000 rpm for 5 minutes prior to assay to remove particulates. Please do not omit this centrifugation step if samples are cloudy and contain particles.
- Severe hemolytic samples should not be used.
- Sample safety: All samples are regarded as potentially infectious and strictly handled in accordance with relevant national standards and guidelines.
- Blood can be collected and tested in this assay using Dried Blood Spot (DBS) samples (see Hemaxis, https://hemaxis.com).

8.3. Assay Procedure (Checklist)

1. Determine the number of 16-well strips needed for the assay and insert them in the frame for current use (for both Spike plate and Background plate). The extra strips are left in the aluminium foil bags with 2 silica gel minibags and stored at 4°C.

   NOTE: Mark the Spike plate strips with a dark marker and the Background plate strips with a red marker.

   Remaining 16-well strips coated with Spike protein of SARS-CoV-2 or Background when opened can be stored in the presence of 2 silica gel minibags at 4°C for up to 1 month.

2. Add 100 µl of Negative control (Negative) and 100 µl of Positive control (Positive) in duplicate in wells of each plate Spike and Background (see 8.1. Preparation and Storage of Reagents).
3. Add 100 µl of diluted serum or plasma samples in duplicate in wells of each plate Spike and Background (see 8.1. Preparation and Storage of Reagents and 8.2 Collection, Storage & Dilution).
4. Cover the plate with plastic film and incubate for 1 hour at RT.
5. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
6. Add 100 µl to each well of the diluted anti-human IgG-HRP (HRP 1X) (see 8.1. Preparation and Storage of Reagents).
7. Cover the plate with plastic film and incubate for 30 min at RT.
8. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
9. Add 100 µl to each well of TMB substrate solution (TMB).
10. Allow the color reaction to develop at RT in the dark for 20 minutes. Do not cover the plate.
11. Stop the reaction by adding 100 µl of Stop Solution (STOP). Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution (STOP) is added.

    ! CAUTION: CORROSIVE SOLUTION !

12. Measure the OD at 450 nm in an ELISA reader.

9. Typical Data

To measure the value of controls and samples, net average optical densities (Net ODs) of each controls and samples should be calculated by subtracting the average ODs of the Background plate to the average ODs of the Spike plate.

Samples	OD Spike Plate	OD Background Plate	Net OD (Spike-Background)
Negative Control	0.108	0.090	0.018
Positive Control	**1.287**	0.040	**1.247**
Serum Healthy Patients	0.139	0.116	0.023
	0.154	0.139	0.015
Serum COVID-19 Patients	**1.33**	0.155	**1.175**
	2.72	0.172	**2.548**
	2.17	0.115	**2.055**

Note: *All PCR positive samples (>35) tested with our Serological Kit were confirmed positive.*

10. Performance Characteristics

Intra-assay: Four serum samples were assayed in replicates 6 times to test precision within an assay.

Inter-assay: Three serum samples were assayed in 4 separate assays to test precision between assays.

Intra-assay	
Samples	**CV (%)**
1	3.31
2	9.86
3	10.49
4	7.14
Inter-assay	
Samples	**CV (%)**
1	12.64
2	6.08
3	9.05

No validated cut-offs are proposed due to low number of serum/plasma tested, but since background values of samples are removed using the Background plate, the COVID-19 negative samples should be close to 0 (<0.1) and the COVID-19 positive should be >0.2. In between the results should be considered as equivocal.

11. Technical Hints, Precautions and Safety

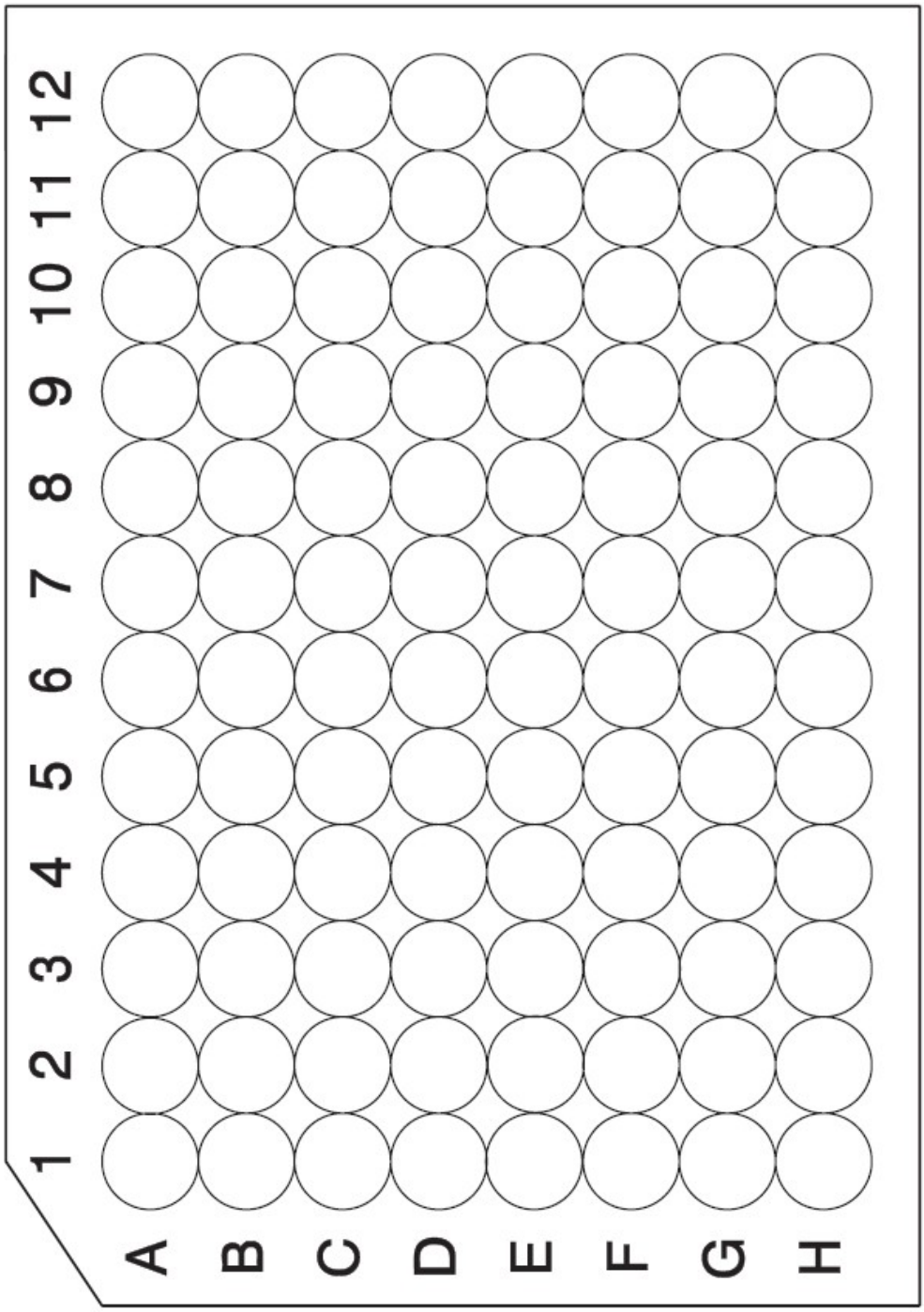
- It is recommended that samples to be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- The kit should not be used beyond the expiration date on the kit label.
- Reagents from the kit with a volume less than 100 µl should be centrifuged.

- Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
- Crystals could appear in the 5X or 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solution.
- Once reagents have been added to the 16-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep TMB Solution protected from light.
- When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
- The Stop Solution (STOP) consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.
- All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.
- This test is only for qualitative detection.
- In the first week of the onset of the infection with the novel coronavirus (SARS-CoV-2) patients results may be negative for IgG.
- 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 strains may cause a light IgG positive in view of similarities in the nucleocapsid protein, but not in the Spike RBD protein.
- Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.

12. Troubleshooting

PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be followed as indicated in the manual.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of HRP too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.

Plate Layout Template



ABEOMICS Inc.
9853 Pacific Heights Blvd. Suite D.
San Diego, CA, 92121
Tel : 858-263-4982 Fax : 858-247-7052,
info@abeomics.com


ABEOMICS, Inc.
9853 Pacific Heights Blvd, STE D.
San Diego, CA-92121
Email: info@abeomics.com
Website: www.abeomics.com