* This kit is for research use only. Not for use in diagnostic procedures.
* Check and follow the latest version of this protocol posted on the website below.
https://www.cellspect.com/

Significance of measurement

This kit is a research ELISA kit to detect human IgM and IgG antibodies that recognize the spike protein of SARS-CoV-2, the causative virus of Covid-19.

Virus detection can be broadly divided into two approaches: one is to detect the virus itself, such as PCR method, and the other is to detect the immune response that develops during infection (IgG, IgM, IgA). In common infections, IgM is an antibody produced early in the infection and disappears with relief of symptoms. IgG begins to be produced later than IgM and continues to be produced for months after healing. This is known to reduce the symptoms of the next infection or to prevent the onset of the next infection.

Measurement principle

① The immobilized SARS-CoV-2 recombinant spike protein on a plate is reacted with the antibody (hereinafter referred to as anti-spike protein antibody) in a sample that recognizes it.

② After the reaction, the sample is removed by washing.

③ React anti-spike protein antibody in the plate with HRP (horseradish peroxidase)-conjugated anti-human IgM / IgG antibodies.

④ Remove excess HRP-conjugated anti-human IgM / IgG antibodies by washing. Add a chromogenic substrate and measure the absorbance.

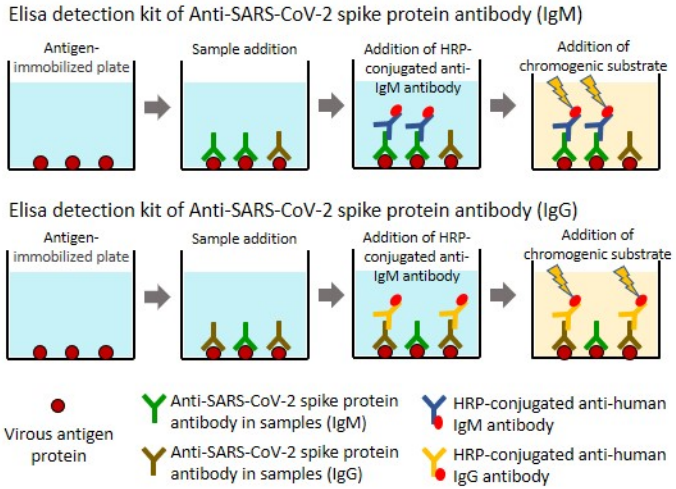

Figure 1 ：Process diagram of measurement

Kit contents

96 tests (product code: RCOEL961)

1.	SARS-CoV-2 spike protein-immobilized plate	×2	
2.	HRP-conjugated anti-human IgM antibody	10 µL×1	●
3	HRP-conjugated anti-human IgG antibody	10 µL×1	●
4	Washing buffer （10× PBST）	50 mL×2	●
5.	Non-fat milk (for blocking buffer)	×1	●
6.	Diluent buffer (10×)	50 mL×1	○
7.	R-1: Chromogenic substrate (TMB)	10 mL×2	Shading bottle
8.	R-2: Stop reagent (1 mol/L hydrochloric acid)	10 mL×2	●
9.	Microplate seal	×2	

Materials required but not supplied

- Microplate reader
- Micropipette and tip
- Multichannel pipette
- Graduated cylinder
- Sample tube
- Microplate shaker
- Paper towel
- Reservoir for multichannel pipette
- Purified water
- 500 mL / 250 mL bottles

Assay protocol

1. Reagents preparation

(1) Preparation of diluent (1×)
Dilute the whole amount of diluent buffer (10×) 10 folds with purified water to make diluent (1×)。
※If precipitation occurs in the diluent buffer (10×), keep it at room temperature and dilute after it completely dissolved.

(2) Preparation of blocking buffer (5% (w/v) non-fat milk solution)
Dissolve the whole amount of non-fat milk (for blocking buffer) in 220 mL of diluent (1×).
※Diluent is prepared immediately before use.
※If the plate is used dividedly, calculate the required amount, dissolve the weighted non-fat milk with Diluent (1×) and prepare a 5% (w/v) non-fat milk solution.

(3) Preparation of antibody/sample diluent (2% (w/v) non-fat milk solution)
Add 240 mL of diluent (1×) to 160 mL of blocking buffer and mix.

(4) Preparation of WR (Working Reagent: HRP-conjugated anti human IgM/IgG antibodies reagent). Dilute 1: 30,000 with antibody / sample diluent.

Table 1. Dilution examples

Test tube No.	Diluted sample	Amount (µL)	Antibody/sample diluent (µL)	Initial ratio	Dilution ratio
1	HRP-conjugated anti-human IgM / IgG antibodies	1	299	1	300
2	Test tube No.1	80	7920	300	30,000

(5) Preparation of WB (Wash Buffer)
Dilute the whole amount of washing buffer (10× PBST) 10 folds with purified water to make WB: Wash Buffer.
※If precipitation occurs in the wash buffer (10× PBST), keep it at room temperature and dilute after it completely dissolved.

2. Blocking of SARS-CoV-2 spike protein-immobilized plate
(1) Remove the SARS-CoV-2 spike protein immobilized-plate from the aluminum pouch and wash with WB.
※Washing process
A) Add 200 µL of WB to each well.
B) Discard the WB in the well after 30 seconds.
C) Repeat A) and B) as described.
D) Drain the solution completely by tapping the plate on a stack of paper towels.
(2) Dispense 200 µL of blocking buffer into each well of the antibody-immobilized plate, and leave it for 1 hour at room temperature.
(3) Discard the blocking buffer and wash twice with WB. (Refer to 2.(1)※Washing process)

3. Preparation of test sample
Dilute the sample 1: 200~1:2000 with antibody/sample diluent and use it as the test sample.

Table 1. Dilution examples of test samples (1:2000)

Test tube No.	Diluted sample	Amount (µL)	Diluent (µL)	Initial ratio	Dilution ratio
1	Serum	10	190	1	20
2	Test tube No.1	5	495	20	2,000

4. Measurement
(1) Dispense 100 µL of prepared test sample into each well of the plate after blocking.
(2) Attach the microplate seal to the plate and allow it to react at room temperature for 1 hour.
(3) After the reaction of (2), discard the reaction solution and wash with WB 5 times. (Refer to 2.(1)※Washing process)
(4) Add 80 µL of WR to each well.
(5) Attach the microplate seal to the plate and allow it to react at room temperature for 1 hour.
(6) After the reaction of (5), discard the reaction solution and wash with WB 5 times as in (3). (Refer to 2.(1)※Washing process)
(7) Add 100 µL of R-1 to each well.
(8) Shake the plate with a microplate shaker. Allow the plate to react at room temperature for 6 minutes and protected from light.
(9) After the reaction of (8). Add 100 µL of R-2 to each well and measure the absorbance at 450 nm.

Notes

1. Regarding test samples:
- Please use fresh samples or samples stored at -20 ° C or below.
- Do not use preservatives.

2. Regarding measurement
- Do not use reagents of different lots.
- Do not expose chromogenic substrate to light.
- Do not let antibody-immobilized plate dry after washing until measurement is completed.
- The bottom surface of plate is immobilized with spike protein which might be shed by contact with the pipette. Please make sure the pipette does not touch the bottom or wall of the plate.
- Unevenness of temperature in plate may cause variations in measured values.
  A) Be sure to bring reagents and plates to room temperature (20~25℃) prior to use
  B) Always perform reactions at room temperature. Indoor places may also have temperature unevenness due to wind blowing or so. Please do not use this kit in places where hot air or cold air may occur, such as the vicinity of air outlet of air conditioner, places closed to windows, etc.
  C) Long time of touch may heat the plate by body temperature and results in a temperature difference in the plate. Please do not touch the plate as possible.
- Please add the reagents in the exact chronological order and keep the reaction time accurately.
- The stop solution is a strong acid. Please handle it with care.

3. Precautions when the kit is used dividedly
- Do not repeat freezing and thawing of the HRP-conjugated anti-human IgM and IgG antibodies supplied with this kit.

- If reagents other than HRP-conjugated anti-human IgM/IgG antibodies are not used during the same day, close the cap tightly and store it in a refrigerator.
- Store the prepared blocking buffer in a refrigerator and use it up within 24 hours.
- Store unused well strips of the plate in a chucking bag with desiccant in a refrigerator.
- HRP-conjugated anti-human IgM/IgG antibody, reagents other than blocking buffer, unused well strips of the plate can be stored in a refrigerator for one week. .

Product Specifications

Number of tests: 96 tests
Measurement method: Indirect ELISA
Measurement wavelength: 450 nm
Measurement sample: Serum
Species reactivity: Human
Storage temperature: Keep HRP-conjugated anti-human IgM/IgG antibody at or below -20 ° C.
Keep other reagents and immobilized plate at 2-8 ° C

References

1.) Keiichi Hiramatsu, Standard Textbook of standard microbiology, 11th edition, Medical study (2012)
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4.) Li Guo, Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19), Clin Infect Dis. 2020 Mar 21.
5.) Juanjuan Zhao, Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019, Clin Infect Dis. 2020 Mar 21.

Manufacturer and distributor

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※QuaResearch is the name of the reagent kit of Cellspect Co., Ltd.

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※ This kit is for research use only. Not for use in diagnostic procedures.
※ For the latest information on products such as instruction manuals, measurement protocols, etc., please check the support corner of our website below.
https://www.cellspect.com/
※ This product is for research use. Please understand that its value cannot be fully guaranteed.
※ The indicated performance is a standard value when a general-purpose microplate reader is used. Please understand that variation may occur depending on the types of equipment.
※ When making inquiries regarding quality, please confirm the Lot No. attached to the packaging bag of reagent kits and contact us.
※ Product specifications, service, packaging form, and measurement protocols may be changed without notice. Please follow this instruction manual properly.
※ Please follow the attached Safety Data Sheet (SDS) for transportation, handling, processing, and disposal of this product.