

\* 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 IgA antibody that recognizes the spike protein (S1) 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). IgA is particularly abundant in mucous membranes such as saliva and the nasopharynx, and generally has the function of infection prevention.

### Measurement principle

- ① The SARS-CoV-2 recombinant spike protein (S1) immobilized on the plate is recognized and reacted with the antibody (hereinafter, abbreviated as anti-spike protein antibody) in a sample.
- ② After the reaction, the sample is removed by washing.
- ③ The anti-spike protein antibody is reacted with HRP (Horseradish peroxidase)-conjugated anti-human IgA antibody.
- ④ Remove excess HRP-conjugated anti-human IgA antibody by washing. Add a chromogenic substrate and measure the absorbance.

### Detection of Anti-SARS-CoV-2 spike protein antibody (IgA)

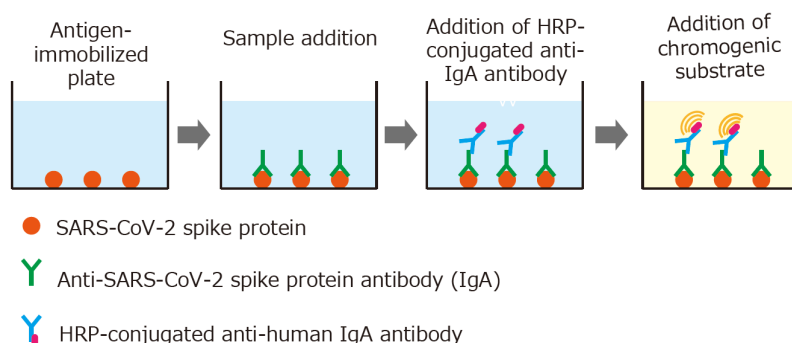


Figure 1 : Process diagram of

### Kit contents

96 tests (product code: RCAEL961-S1)			
1.	SARS-CoV-2 spike protein (S1)-immobilized plate	×1	
2.	HRP-conjugated anti-human IgA antibody	10 µL×1	●
3.	Wash buffer (10× PBST)	100 mL×1	●
4.	Bovine serum albumin (for diluent buffer)	×1	●
5.	Diluent buffer (10×)	50 mL×1	○
6.	R-1 : Chromogenic substrate (TMB)	22 mL×1	Shading bottle
7.	R-2 : Stop reagent (1 mol/L hydrochloric acid)	22 mL×1	●
8.	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 antibody/sample diluent (1% (w/v) bovine serum albumin solution)  
Add 500 mL of diluent (1×) to bovine serum albumin and mix.
  - (3) Preparation of WR (Working Reagent: HRP-conjugated anti human IgA antibody reagent).  
Dilute 1: 100,000 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 IgA antibody	5	495	1	100
2	Test tube No.1	30	29970	100	100,000

(4) 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. Preparation of test sample

Dilute the sample 1: 200~1:2000 with diluent (1×) and use it as the test sample.

Table 1. Dilution examples of test samples (1:1000)

Test tube No.	Diluted sample	Amount (μL)	Diluent (μL)	Initial ratio	Dilution ratio
1	Test sample*	10	190	1	20
2	Test tube No.1	10	490	20	1,000

\* When using saliva or sputum as the test sample, centrifuge at 3000 rpm, 4°C, 15 minutes and use the supernatant.

3. Measurement

(1) Remove the SARS-CoV-2 spike protein-immobilized plate from the aluminum pouch and wash it once 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 100 μL of prepared test sample into each well of the plate after blocking.

(3) Attach the microplate seal to the plate and allow it to react at room temperature for 1 hour.

(4) After the reaction of (3), discard the reaction solution and wash with WB 5 times. (Refer to ※Washing process)

(5) Add 100 μL of WR to each well.

(6) Attach the microplate seal to the plate and allow it to react at room temperature for 1 hour.

(7) After the reaction of (6), discard the reaction solution and wash with WB 5 times as in (4). (Refer to ※Washing process)

(8) Add 100 μL of R-1 to each well.

(9) Shake the plate with a microplate shaker. Allow the plate to react at room temperature for 10 minutes and protected from light.

(10) After the reaction of (9). 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°C) 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 IgA antibody supplied with this kit.
- If reagents other than HRP-conjugated anti-human IgA antibody 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 IgA antibody, reagents other than blocking buffer, unused well strips of the plate can be stored in a refrigerator for one week.

Product Specifications

Immobilized protein: Spike protein (S1)

Number of tests: 96 tests

Measurement method: Indirect ELISA

Measurement wavelength: 450 nm

Measurement sample: Serum, saliva, sputum

Species reactivity: Human

Immobilized antigen: SARS-CoV-2 spike protein (S1 domain, E. coli expression system)

Storage temperature: Store at 4° C.

Expiration date: 12 months after production.

## References

- 1.) Keiichi Hiramatsu, Standard Textbook of standard microbiology, 11th edition, Medical study (2012)
- 2.) Andrea Padoan, IgA-Ab response to spike glycoprotein of SARS-CoV-2 in patients with COVID-19: A longitudinal study, Clinica Chimica Acta, 507, 164-166, Aug., 2020.
- 3.) Yin Xia Chao, The role of IgA in COVID-19, Brain, Behavior, and Immunity, 87, Jul., 2020 .

## Manufacturer and distributor

Cellspect Co., Ltd.

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

## Contact information

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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.