

- * This kit is for research use only. Not for use in diagnostic procedures.
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<https://www.cellspect.com/>

Significance of measurement

This kit is a research ELISA kit to detect human IgM and IgG antibodies that recognize the nucleocapsid 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 SARS-CoV-2 recombinant nucleocapsid protein on an immobilized plate is reacted with an antibody (hereinafter referred to as human IgM/IgG) in a sample that recognizes it.
- ② After the reaction, the sample is removed by washing.
- ③ React with human IgM/IgG on immobilized plates 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.

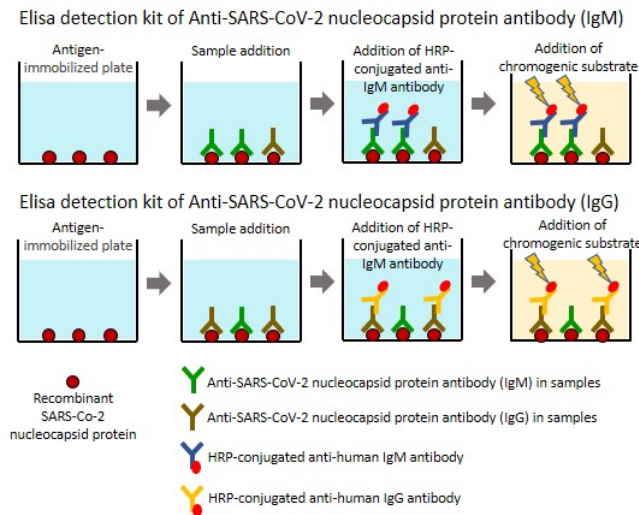


Figure 1 : Process diagram of measurement

Kit contents

96 tests (product code: ERCOEL961-N)			
1.	SARS-CoV-2 nucleocapsid 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.	Wash buffer (10× PBST)	100 mL×1	●
5.	Bovine serum albumin (for diluent)	×1	●
6.	Diluent buffer (10×)	50 mL×1	○
7.	R-1 : Chromogenic substrate (TMB)	22 mL×1	Shading bottle
8.	R-2 : Stop reagent (1 mol/L hydrochloric acid)	22 mL×1	●
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 50mL of diluent buffer (10 x) 10 folds with purified water to make diluent (1x).
※If precipitation occurs in the diluent buffer (10x), keep it at room temperature to completely dissolve it and then dilute it.
 - (2) Preparation of antibody/sample diluent (1% (w / v) bovine serum albumin solution)
Add 500 mL of diluent (1x) to bovine serum albumin and mix them well.

- (3) Preparation of WR (Working Reagent: HRP-conjugated anti IgM/IgG 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 IgM/IgG antibody	4	996	1	250
2	Test tube No.1	5	1995	250	100,000

- (4) Preparation of WB (Wash Buffer)
Dilute 50mL of wash buffer (10x) 10 folds with purified water to make WB: Wash Buffer.
※If precipitation occurs in the wash buffer (10x), keep it at room temperature to completely dissolve it and then dilute it.

2. Preparation of test sample
Dilute the sample 1: 200~1:2,000 with antibody/sample diluent and use it as the test sample.

Table 2. Dilution examples of test samples (1 : 1,000)

Test tube No.	Diluted sample	Amount (μL)	Antibody/sample diluent (μL)	Initial ratio	Dilution ratio
1	Serum	4	1996	1	500
2	Test tube No.1	1000	1000	500	1,000

3. Measurement
- (1) Remove the SARS-CoV-2 nucleocapsid 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) specific times.
D) Drain the solution completely by tapping the plate on a stack of paper towels.
- (2) Dispense 100 μL of the test sample into each well of the immobilized plate.
- (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 3 times. (see the 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 3 time as in (4). (see the 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

- Regarding test samples:
 - Please use fresh samples or samples stored at -20 ° C or below.
 - Do not use preservatives.
- Regarding measurement
 - Do not use reagents of different lots.
 - Do not expose chromogenic substrate to light.
 - Do not let immobilized plate dry after washing until measurement is completed.
 - The bottom surface of plate is immobilized with nucleocapsid 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.
 - Be sure to bring reagents and plates to room temperature (20~25°C) prior to use.
 - 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.
 - 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.
- Precautions when using divided kit
 - If reagents are not used during the same day, keep the cap tightly closed and stored in a refrigerator.
 - Store unused well strips of the immobilized plate in a chocking bag with desiccant in a refrigerator.
 - HRP-conjugated anti-human IgM/IgG antibodies and unused well strips of immobilized plate can be stored in the refrigerator for one week after opening.

Product Specifications

Number of tests: 96 tests

Instruction manual

Measurement method: Indirect ELISA
Measurement wavelength: 450 nm
Measurement sample: Serum
Species reactivity: Human
Storage temperature: Store at 2-8 °C
Expiration date: This product is valid for 12 months after production.

References

- 1.) Keiichi Hiramatsu, Standard Textbook of standard microbiology, 11th edition, Medical study (2012)
- 2.) Anu Haveri, Serological and molecular findings during SARS-CoV-2 infection: the first case study in Finland, January to February 2020, Eurosurveillance. Volume 25, Issue 11, 19, Mar 2020.
- 3.) Wanbing Liu, Evaluation of Nucleocapsid and Spike Protein-based ELISAs for detecting antibodies against SARS-CoV-2, J Clin Microbiol. 2020 Mar 30.
- 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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※Quoresearch 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 reagent kit packaging bag 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, disposal of this product.