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SARS-CoV-2 Anti-RBD Antibody Profiling Kit

Infection by the novel coronavirus is initiated when a spike protein on the surface of the virus binds to the human cell surface receptor ACE2*. Virus infection prompts the production of antibodies against the spike protein. Some anti-spike (RBD**) antibodies neutralize the virus while the others do not neutralize the virus (non-neutralizing antibodies). Among non-neutralizing antibodies, there are some antibodies which enhance the severity of the disease. Therefore, knowing the type of antibodies in clinical samples is critical for the prevention and treatment of the disease.[1)] This kit is used for profiling anti-RBD antibodies in clinical samples. The assay design takes advantage of the interaction between the RBD and ACE2. The assay does not require presence of the virus or cells, allowing for simple and rapid testing of a large number of clinical samples.

*ACE2: angiotensin-converting enzyme 2

**RBD: Receptor Binding Domain

Component

Name	Materials	Quantity
ACE2 coated microplate	Microwell strips coated with ACE2 recombinant protein	8-well × 12 strips
Positive control	Human derived monoclonal antibody (IgG)	200 µL × 1 vial
Reaction buffer	Buffer for dilute Positive controls, samples, and RBD concentrate (Ready-to-use)	50 mL × 1 bottle
Wash concentrate (10x)	Buffer for washing microwells (10x)	100 mL × 1 bottle
RBD concentrate	His tagged RBD protein (1,000x)	50 µL × 1 vial
Conjugate diluent	Buffer for diluting HRP conjugated antibody (Ready-to-use)	20 mL × 1 bottle
HRP conjugated antibody	HRP conjugated anti-His-tag monoclonal antibody (101x)	150 µL × 1 vial
Substrate solution	TMB/H_2O_2 solution (Ready-to-use)	20 mL × 1 bottle
Stop solution	0.5N H_2SO_4 solution (Ready-to-use)	20 mL × 1 bottle
Primary reaction microplate	96-well micro plate for liquid-phase reaction (U bottom, non-treated, polystyrene)	1 plate
Plate seals	Plate seals	3 pieces

Speculated reaction model

RBD antibodies are absent. Signal is generated by the interaction between RBD and ACE2.

Neutralizing antibodies are predominant. RBD-ACE2 interaction is inhibited by samples with a high neutralizing activity, resulting in a reduced signal.

Non-neutralizing antibodies are predominant. RBD is crosslinked by anti-RBD antibodies, which facilitates the binding of RBD to ACE2. As a result, the signal is enhanced compared with samples with no anti-RBD antibodies.

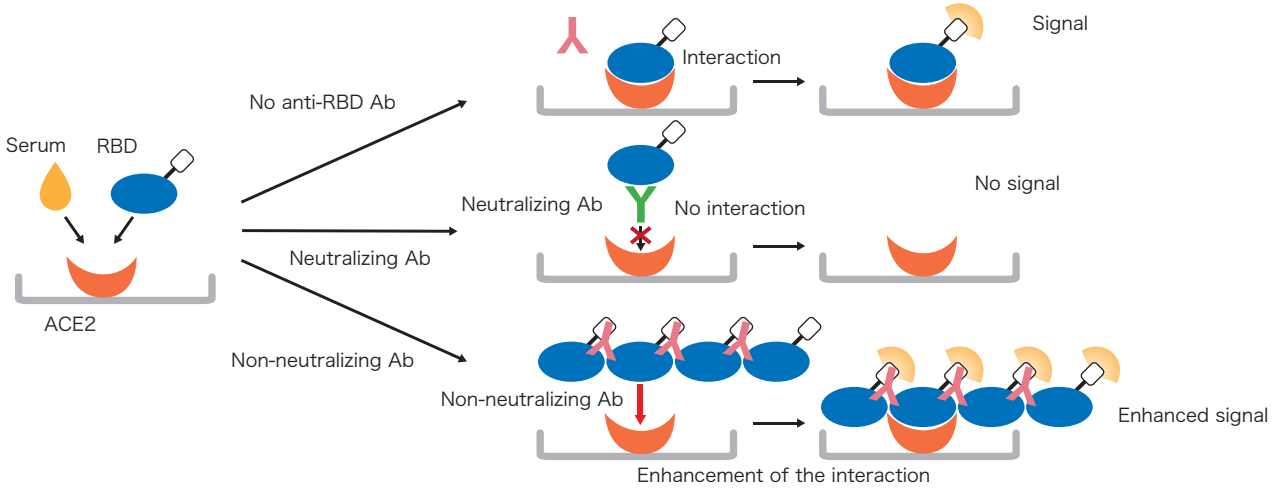


Figure 1. Speculated reaction model

Assay Principle

The assay is based on the interaction between ACE2 immobilized on the plate and His-tagged RBD (Figure 1). The effect of antibodies on the ACE2-RBD interaction is assessed by comparing to the base-line binding between ACE2 and RBD in the blank (antibodies are absent).

Calculation of Inhibition Rate

The RBD-ACE2 binding inhibition rate is calculated using the following formula:

$$\text{Inhibition Rate (\%)} = \left(1 - \frac{\text{ABS Sample}}{\text{ABS Blank}}\right) \times 100$$

Example 1 (Patient-derived monoclonal antibodies)

Purpose

The kit was evaluated using 20 monoclonal antibodies* isolated from patients infected with the novel coronavirus, and the results were compared with those of a virus neutralization test.

Procedure

Monoclonal antibody profiling

A dilution series of each monoclonal antibody were prepared and assayed using the kit, and the inhibition rate was calculated.

Virus neutralization test

The test was performed by Keio University School of Medicine according to the protocol established by National Institute of Infectious Diseases.

Results

Based on the measurements of the dilution series of the monoclonal antibodies with the kit, the pattern of inhibition of RBD-ACE2 interaction fell into two groups: those with a positive inhibition rate (the signal was decreased) and those with a negative inhibition rate (the signal was enhanced) (Figure 2). Compared with the results of virus neutralization test performed by the Keio University School of Medicine (Table 1), monoclonal antibodies with a high neutralizing activity had a high inhibition rate as measured with the kit. All monoclonal antibodies with no neutralizing activity in the virus neutralization test had a negative inhibition rate (signal enhancement).

*Monoclonal antibodies were isolated from patients at the Keio University School of Medicine.

Table 1. Results of virus neutralization test of the monoclonal antibodies isolated from patients

	MNT#	mAb
	<3.125	mAb 1, mAb 2, mAb 3, mAb 4
	6.25	mAb 5
	25	mAb 6
	50	mAb 7
	100	mAb 8, mAb 9
	>100	mAb 10, mAb 11, mAb 12, mAb 13, mAb 14, mAb 15, mAb 16, mAb 17
	ND	mAb 18, mAb 19, mAb 20

The levels of neutralizing activity of the antibodies are color-coded in the table.

#MNT: Minimum neutralization concentration with the microneutralization test (μg/mL)

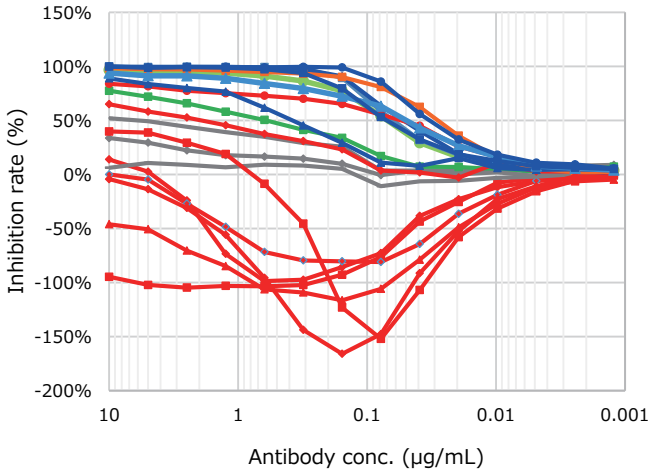


Figure 2. Profiles of the monoclonal antibodies isolated from patients

The colors of the lines in the graph correspond to those for the neutralization activities in the virus neutralization test results shown in Table 1.

Dilution series (14 concentrations): concentrations of monoclonal antibodies (μg/mL)

10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.0781, 0.0391, 0.0195, 0.00977, 0.00488, 0.00244, 0.00122

Example 2 (Mixed samples with neutralizing and non-neutralizing monoclonal antibodies)

Purpose

Mixed samples containing both neutralizing and non-neutralizing monoclonal antibodies were used to assess the assay performance of the kit when both types are present in a sample.

Procedure

Samples were prepared by mixing neutralizing and non-neutralizing monoclonal antibodies (mAb 3) at various ratios (Table 2).

Two mixed samples were prepared, one with a high neutralizing activity (mAb 1) and the other with a moderate neutralizing activity (mAb 2).

A dilution series of each mixed sample listed in Table 2 were prepared, and concentration-dependent changes in the inhibition rate of RBD-ACE2 interaction were measured.

Table 2. Preparation of mixed samples containing neutralizing and non-neutralizing monoclonal antibodies

A) Mix sample composed of mAb 1 and mAb 3

mAb 1	100%	80%	60%	40%	20%	10%	5%	0%
mAb 3	0%	20%	40%	60%	80%	90%	95%	100%

B) Mix sample composed of mAb 2 and mAb 3

mAb 2	100%	80%	60%	40%	20%	0%
mAb 3	0%	20%	40%	60%	80%	100%

Virus-neutralizing activity (minimum antibody concentration that neutralizes the virus) of the monoclonal antibodies in the mixed samples

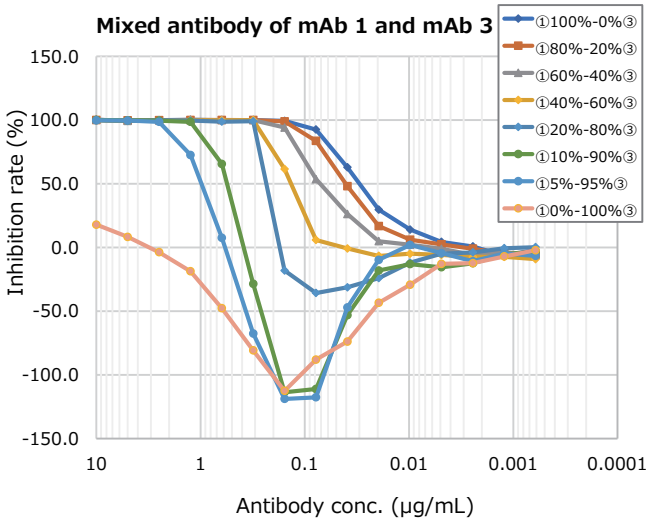
Neutralizing antibody: mAb 1, < 3.125 μg/mL; mAb 2, 25 μg/mL

Non-neutralizing antibody: mAb 3, > 100 μg/mL

Results

A) For samples containing a monoclonal antibody with a high neutralizing activity, signal was enhanced only when the content of the neutralizing antibodies was 20% or less.

B) For samples containing the antibody with a moderate neutralizing activity, signal was enhanced when the content of the non-neutralizing antibodies was 20% or more.



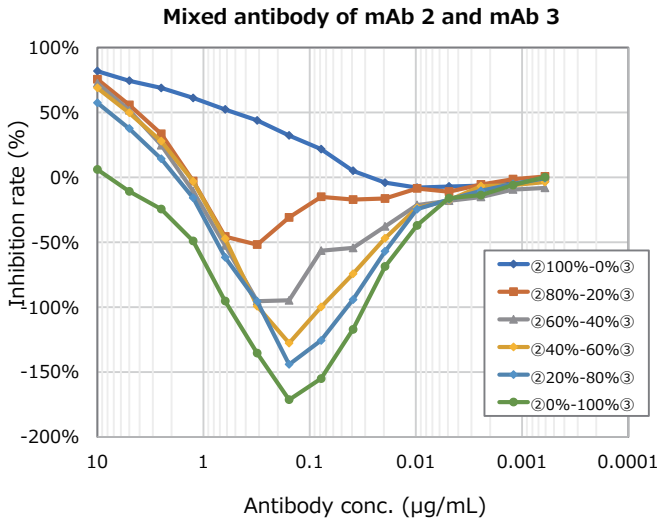


Figure 3. Profiles of the mixed samples containing neutralizing and non-neutralizing monoclonal antibodies

Dilution series (15 concentrations): monoclonal antibody concentrations (μg/mL)

10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.0781, 0.0391, 0.0195, 0.00977, 0.00488, 0.00244, 0.00122, 0.00061

Example 3 (Sera from patients)

Purpose

Dilution series of sera from patients and healthy donors were measured to assess the assay performance of the kit with clinical samples.

Procedure

Preparation of sample dilution series

Dilution series of sera from 7 patients infected with novel coronavirus and 2 healthy donors were prepared using a dilution buffer. (Dilution factor: 10x, 20x, 40x, 80x, 160x, 320x, 640x)

Profiles of the clinical samples

Inhibition rates were calculated based on the measurements of the above dilution series with the kit. The inhibition rates were plotted against the dilution factors (Figure 4).

Virus neutralization test

The test was performed by Keio University School of Medicine according to the protocol established by National Institute of Infectious Diseases.

Results

Sera from 2 healthy donors: Inhibition rate was nearly 0%, regardless of dilution. No effect on RBD-ACE2 interaction was observed.
Patient serum A*: Inhibitory effect was observed with the dilution factors of 10x through 40x, and signal enhancement was observed with the dilution factors of 80x and 160x.
Patient sera B, C, D: Dilution factor-dependent inhibitory effect on RBD-ACE2 interaction was observed.
Patient sera E, F, G: Dilution factor-dependent enhancement of RBD-ACE2 interaction was observed.

*Both neutralizing and non-neutralizing monoclonal antibodies were found among the monoclonal antibodies isolated from patient A by Keio University School of Medicine (Table 3).

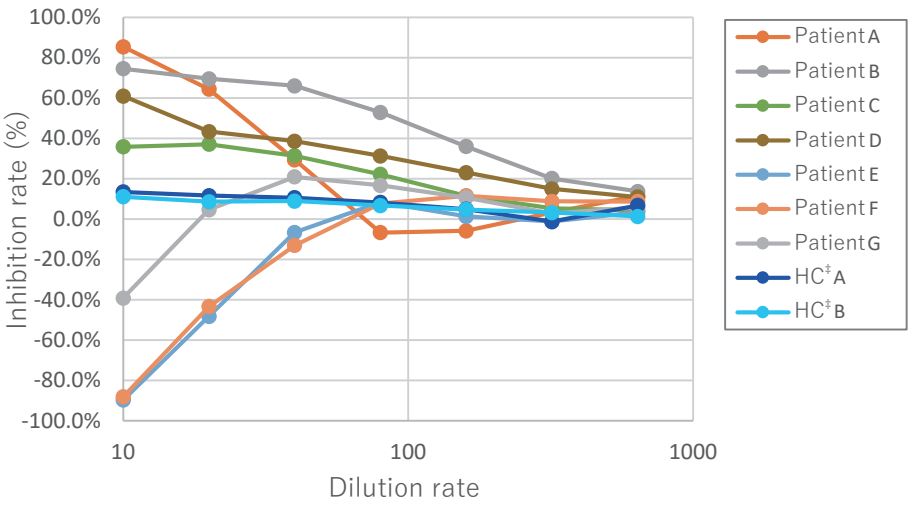


Figure 4. Profiles of clinical samples

‡HC: Healthy Control

Table 3. Virus-neutralizing activity of monoclonal antibodies derived from patient A and the profiles determined using the kit

mAb	Virus NT test†	Inhibition rate
mAb 1	50	97%
mAb 3	>100	14%
mAb 4	>100	0
mAb 5	>100	-46%
mAb 6	>100	-95%
mAb 2	ND	34%
mAb 7	ND	ND
mAb 8	ND	ND

*Inhibition rate with 10 μg/mL of mAb determined using the kit
*ND: Not Determined
†Virus NT test: Virus neutralization test

< Reference>

1) Akiko Iwasaki *et al.*, Nature Reviews Immunology, vol. 20, page 339-341

Product list

Code No.	Product name	Size
5370	SARS-CoV-2 Anti-RBD Antibody Profiling Kit	96 wells

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Produced by
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