SARS-CoV-2 Anti-RBD Antibody Profiling Kit

Code No. 5370

Intended Use

SARS-CoV-2 Anti-RBD Antibody Profiling Kit can be used to evaluate the modification of the interaction between the receptor binding domain (RBD) and ACE2 involving anti-SARS-CoV-2 antibodies in serum.

Individual users should determine appropriate conditions when using other types of samples.

This assay kit is for research use only and not for use in diagnostic or therapeutic procedures.

Storage

- Upon receipt store all components at 2-8°C.
- Don't expose reagents to excessive light.

Introduction

The novel coronavirus, SARS-CoV-2, binds via spikes on its surface to the receptor ACE2 on human cells, and infects to human. SARS-CoV-2 infection induces production of anti-spike (RBD*) antibodies. On the other hand, there are some types of anti-RBD antibodies such as neutralizing antibodies, non-neutralizing antibodies, and antibodies involved in increasing severity of COVID-19. Therefore, it is very important to know what types of antibody is present in the blood in the prevention and treatment of SARS-CoV-2 infection.

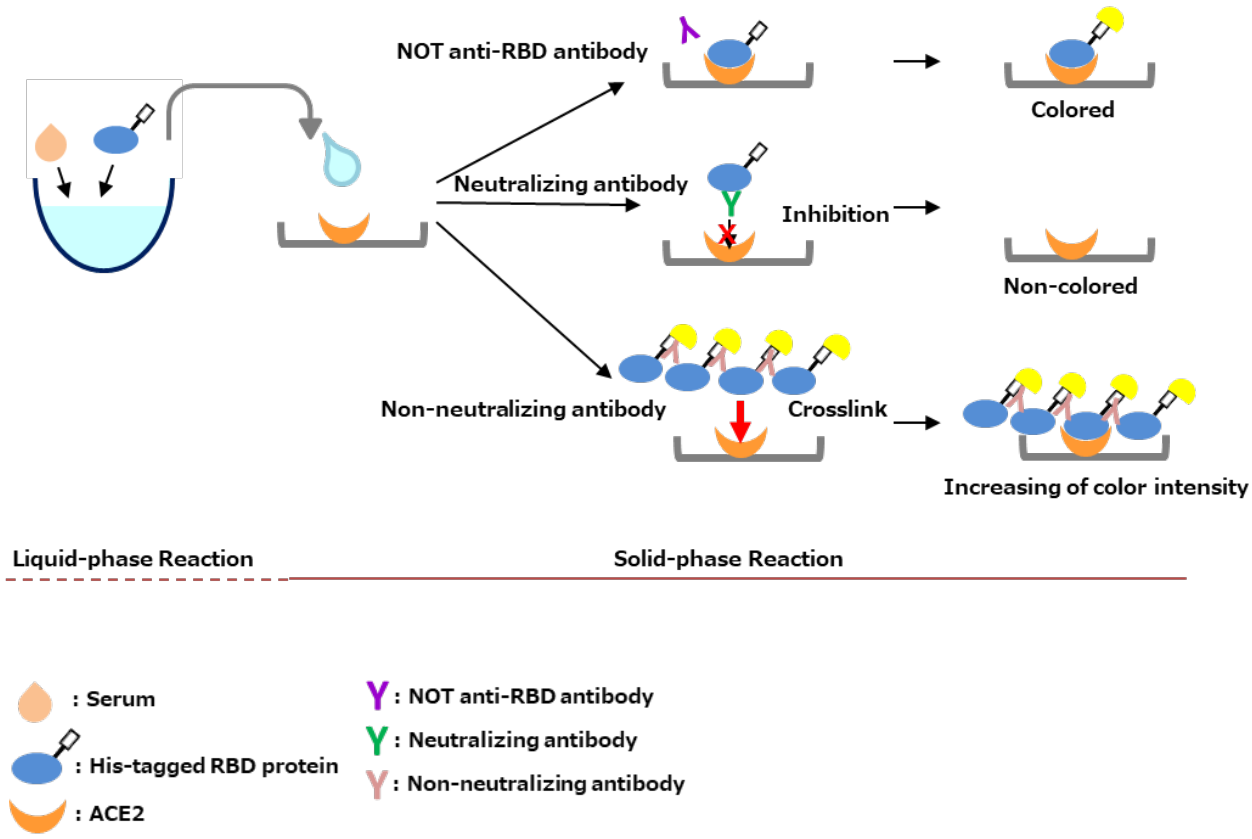
SARS-CoV-2 Anti-RBD Antibody Profiling Kit allows profiling of anti-RBD antibodies in serum.

*RBD: Receptor Binding Domain. The binding region to ACE2.

Principle of the Assay

SARS-CoV-2 Anti-RBD Antibody Profiling Kit consists of two reaction systems, a liquid-phase reaction and a solid phase-reaction. ACE2 is immobilized on the microplate. This is an assay system that utilizes the binding of ACE2 and His-tagged RBD. The effect of the anti-RBD antibodies on the ACE2-RBD interaction is evaluated relative to the ACE2-RBD binding level in the blank wells, condition in the absence of antibodies.

Speculated Reaction Models



Materials Provided

All samples and the positive control should be assayed in duplicate. The following components are supplied and are sufficient for the one 96-well microplate kit.

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 diluting 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 (501x)	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 (Non-treated, polystyrene)	1 plate
Plate seals	Plate seals	3 pieces

Materials Required but not Provided

- **Pipettors (single and multichannel):** 2-20 µL, 20-200 µL and 200-1,000 µL precision pipettors with disposable tips.
- **Precision repeating pipettor**
- **Plastic tubes** (1.5 mL, 15 mL etc.)
- **(Optional) Microplate washer:** Manual washing is possible but not preferable.
- **Reagent reservoirs**
- **Deionized water of the highest quality**
- **Disposable paper towels**
- **Plate reader:** capable of measuring absorbance in 96-well plates at dual wavelengths of 450 nm/620 nm. The plate can also be read at a single wavelength of 450 nm, which will give a somewhat higher reading.
- **(Optional) Software package facilitating data generation and analysis**

Precautions and Recommendations

- All reagents except **RBD concentrate** and **HRP conjugated antibody** need to be brought to room temperature (20-25°C) before use.
- All microwells strips that are not immediately required should be returned to the zip-lock pouch, which must be carefully resealed to avoid moisture absorption.
- Do not use kit components beyond the indicated kit expiration date.
- Do not mix reagents with different batches and kits.
- Do not mouth pipette or ingest any of the reagents.
- Fresh samples should be used. Aliquot each sample and store below -20°C if necessary. Avoid repeated freezing and thawing. Never store the samples at 4°C, as samples might be affected by storage at this temperature.
- The buffers and reagents in this kit may contain preservatives. Care should be taken to avoid direct contact with these reagents. When disposing of it, flush with plenty of water and/or handle it according to the regulations of the facility.
- Dispose tetra-methylbenzidine (TMB) containing solutions in compliance with local regulations.
- Avoid contact with the acidic **Stop solution** and **Substrate solution**, which contains hydrogen peroxide. Protect eyes and skin and handle with care. In case of contact with the **Stop solution** and the **Substrate solution**, wash skin thoroughly with water and seek medical attention, when necessary.
- Wear gloves and eye protection when handling immunodiagnostic materials and samples of human origin, and these reagents. In case of contact with the **Stop solution** and the **Substrate solution**, wash skin thoroughly with water and seek medical attention, when necessary.
- **Biological samples may be contaminated with infectious agents. Do not ingest, expose to open wounds or breathe aerosols. Wear protective gloves and dispose of biological samples properly.**

Sample Collection and Storage

Serum: Use a serum separation tube and separate the serum according to the manufacturer's manual. Fresh samples should be used. Aliquots of serum may also be stored at -20°C or below for extended periods of time. Avoid repeated freeze-thaw cycles.

Other biological samples: MBL has not validated.

Summary of Procedure

Add 100 µL of Positive controls and samples to Primary reaction microplate's wells

↓

Add 100 µL of RBD Reaction Solution to Primary reaction microplate's wells

↓ Incubate for 30 minutes at room temperature (20-25°C).

Transfer 100 µL of the solution from Primary reaction microplate's wells to ACE2 coated microplate's wells

↓ Incubate for 30 minutes at room temperature (20-25°C).

Wash the wells

↓

Add 100 µL of Conjugate Solution

↓ Incubate for 30 minutes at room temperature (20-25°C).

Wash the wells

↓

Add 100 µL of Substrate solution

↓ Incubate for 15 minutes at room temperature (20-25°C).

Add 100 µL of Stop Solution

↓

Measure absorbance at 450 nm

Detailed Protocol

The **SARS-CoV-2 Anti-RBD Antibody Profiling Kit** is provided with removable strips of wells so the assay can be carried out on separate occasions using only the number of strips required for the particular determination. Since experimental conditions may vary, Positive control included in the kit, should be measured in each assay as a guide. Disposable pipette tips and reagent troughs should be used for all liquid transfers to avoid cross-contamination of reagents or samples.

<Preparation of Working Solutions>

All reagents except **RBD concentrate** and **HRP conjugated antibody** need to be brought to room temperature prior to the assay.

1. Wash Buffer
   Prepare "Wash Buffer" by adding 100 mL of the **Wash concentrate (10x)** to 900 mL of deionized (distilled) water (ddH_2O). Mix well.

2. RBD Reaction Solution
   Prepare "RBD Reaction Solution" by diluting **RBD concentrate, 1:500** with **Reaction buffer**. Prepare only a sufficient amount of the ACE2 Reaction Solution just before use (*e. g.,* add 24 µL of **ACE2 concentrate** to 12 mL of **Reaction buffer**).

3. Conjugate Solution
   Prepare "Conjugate Solution" by diluting **HRP conjugated antibody, 1:100** with **Conjugate diluent**. Prepare only a sufficient amount of the Conjugate Solution just before use (*e. g.,* add 120 µL of **HRP conjugated antibody** to 12 mL of **Conjugate diluent**).

4. Other reagents are ready-to-use.

<Preparation of Positive control and samples>

►For screening of neutralizing anti-RBD antibodies and inhibitors of RBD-ACE2 binding.
Prepare positive control and samples as follows:
－Dilute **Positive control** 1:4 with **Reaction buffer**.
(*e. g.,* add 50 µL of **Positive control** to 200 µL of **Reaction buffer**)
－Dilute samples with **Reaction buffer** appropriately. Samples require the proper dilution ratio if necessary.
The **Reaction buffer** is added to the Blank wells, and the Blank wells mean 0% inhibition control.

	1	2	3	…	12
A	Blank	Sample 3	…		
B	Blank	Sample 3	…		
C	Positive control	Sample 4	…		
D	Positive control	Sample 4	…	…	
E	Sample 1	Sample 5	…		
F	Sample 1	Sample 5			
G	Sample 2	…			
H	Sample 2	…			

Note: Do not use a Repeating pipette. Change tips for every dilution.
Since an equal volume of RBD Reaction Solution is added when Liquid-phase Reaction, resulting in the final dilution rate will be further 2-fold (x10 ~ etc.).

►For evaluation of non-neutralizing anti-RBD antibodies using dilution series.
Prepare positive control and samples as follows:
－To prepare the x5 diluted solution, dilute **Positive control** and samples 1:4 with **Reaction buffer**.
(*e. g.,* add 50 µL of **Positive control** and samples to 200 µL of **Reaction buffer**)
－Make a two-fold serial dilution.
(*e. g.,* transfer 125 µL of x5 solution to the next tube filled with 125 µL of **Reaction buffer**)
Mix each tube thoroughly before the next transfer. The **Reaction buffer** is added to the Blank wells, and the Blank wells mean 0% inhibition control.

	1	2	3	…	12
A	Positive control (x5)	Sample 1 (x5)	…		
B	Positive control (x10)	Sample 1 (x10)	…		
C	Positive control (x20)	Sample 1 (x20)	…		
D	Positive control (x40)	Sample 1 (x40)	…		
E	Positive control (x80)	Sample 1 (x80)	…		
F	Positive control (x160)	Sample 1 (x160)			
G	Positive control (x320)	Sample 1 (x320)			
H	Blank	Blank			

Note: Do not use a Repeating pipette. Change tips for every dilution.
Since an equal volume of RBD Reaction Solution is added when Liquid-phase Reaction, resulting in the final dilution rate will be further 2-fold (x10, x20 ~ etc.).

<Assay Procedure>

A. Liquid-phase Reaction

1. Pipette **100 µL** of **Reaction buffer** to the Blank wells. And, pipette **100 µL** of Diluted Positive controls and samples to the wells of **Primary reaction microplate**. (See "Preparation of Positive controls and samples" above.)

2. Add **100 µL** of RBD Reaction Solution to each well.

3. Incubate the plate for 30 minutes at room temperature (20-25°C).

B. Solid-phase Reaction

1. Remove the appropriate number of microtiter wells of **ACE2 coated microplate** from the foil pouch and place them into the well holder. Return any unused wells to the foil pouch, refold, seal with tape and store at 4°C.

2. Transfer **100 µL** of the solution after step A, Liquid-phase Reaction, to the wells of **ACE2 coated microplate**. (See "Preparation of Positive controls and samples" above.)

3. Incubate the plate for 30 minutes at room temperature (20-25°C).

4. Wash the plate 4-times by filling each well with Wash Buffer (350 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or microplate washer. Tap the plate on a paper towel to remove any remaining solution.

5. Pipette **100 µL** of Conjugate Solution to the wells of **ACE2 coated microplate** with a multichannel pipet.

6. Incubate the plate for 30 minutes at room temperature (20-25°C).

7. Wash the plate 4-times as in step 4.

8. Pipette **100 µL** of **Substrate solution** to the wells of **ACE2 coated microplate** with a multichannel pipet.

9. Incubate the plate for 15 minutes at room temperature (20-25°C).

10. Add **100 µL** of **Stop solution** to the wells of **ACE2 coated microplate** with a multichannel pipet.

11. Measure absorbance in each well using a spectrophotometric microplate reader at dual wavelengths of 450/620 nm. The primary wavelength is 450 nm, and the reference wavelength is 620 nm. Read the microplate at 450 nm if only a single wavelength can be used. Wells must be read within 20 minutes of adding the Stop solution.

Note-1: Each solution and reagent should be used at room temperature (20-25°C).
Note-2: Use new disposable tips, reservoirs and paper towels at each step to avoid contamination.
Note-3: Once the solution has been poured into the reservoir, never return it to the bottle.
Note-4: Complete removal of liquid at each step is essential to good performance.
Note-5: Ensure that the back of the plate is clean and dry, and that no air bubbles are present on the surface of the liquid in the wells before reading.
Note-6: The incubation time for color development may vary depending on the environment such as temperature. It can be adjusted according to the coloring intensity.

Calculation of Results

Average the duplicate readings of each diluted Positive control and sample. Then, the inhibition rate of each sample can be calculated by the following formula.

$$\text{Inhibition rate (\%)} = \left(1 - \frac{\text{O.D. value of Sample}}{\text{O.D. value of Blank}}\right) \times 100$$

Quality Control

To assure the validity of the results, each assay must include both Positive control and Blank (0% inhibition control). The net O.D. values (450 nm) of these controls must fall within the ranges listed as below. If O.D. values do not meet the requirements, the assay is invalid and should re-assay.

O.D. values of Blank (0% inhibition control) > 0.5
Inhibition rate of Positive control (final dilution rate is x10) > 50%

Troubleshooting

1. Do not dry the plate as it can adversely affect the assay results. Immediately add each solution step by step.

Reagent Stability

All kit components must be stored at 2-8°C. Product explanation date is described in kit box.

Assay Characteristics

1. Precision

Intra-assay precision and Inter-assay precision were assessed by using samples (monoclonal antibodies) known their concentration and inhibition level.
(Sample 1: a monoclonal antibody with neutralizing activity, Sample 2: a monoclonal antibody with neutralizing activity, Sample 3: a monoclonal antibody with non-neutralizing activity)

Intra-assay Precision (Precision within an assay)

Samples were tested in septuplicate on one plate to assess intra-assay precision.

• Intra-assay (Within-Run, n=7)

Inhibition Rate

	Sample 1	Sample 2	Sample 3
1	93.9%	75.8%	-164.8%
2	94.1%	76.3%	-162.7%
3	94.4%	77.5%	-150.0%
4	95.9%	76.3%	-147.6%
5	94.6%	75.0%	-149.4%
6	94.6%	75.8%	-152.4%
7	94.5%	76.8%	-151.4%
MEAN	**94.5%**	**76.2%**	**-154.0%**
S.D.	**0.6%**	**0.8%**	**6.8%**
C.V.	**0.7%**	**1.1%**	**-4.4%**

Inter-assay Precision (Precision between assays)

Six septuplicate-measurements were performed to assess inter-assay precision.

• Inter-assay (Run-to-Run, n=6)

Inhibition Rate

	Sample 1	Sample 2	Sample 3
1	96.0%	73.1%	-143.3%
2	96.1%	73.7%	-134.1%
3	96.8%	76.4%	-155.7%
4	94.5%	76.2%	-154.0%
5	95.1%	75.1%	-169.5%
6	95.9%	78.1%	-190.4%
MEAN	**95.7%**	**75.4%**	**-157.8%**
S.D.	**0.8%**	**1.9%**	**19.9%**
C.V.	**0.8%**	**2.5%**	**-12.6%**

Examples of Test Results

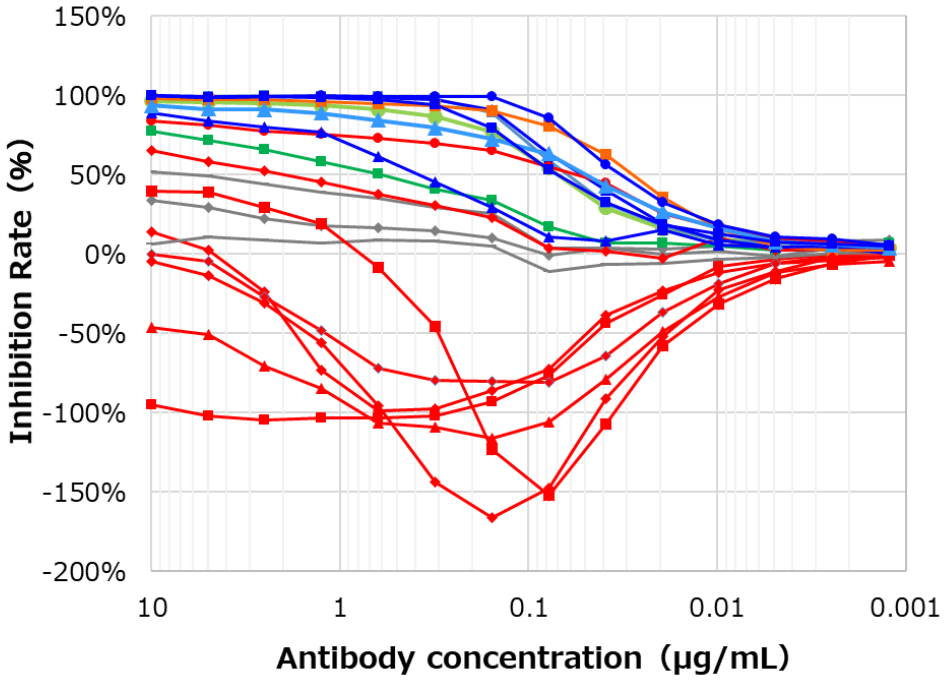


Fig.1 Inhibitory behavior of monoclonal antibodies in the dilution series.
The monoclonal antibodies were isolated from COVID-19 patients. (n =20) The colors on the plot lines match the colors in the classification shown in Table 1.

	MNT	Samples (monoclonal antibody)
	<3.125	mAb1, mAb2, mAb3, mAb4
	6.25	mAb5
	25	mAb6
	50	mAb7
	100	mAb8, mAb9
	>100	mAb10, mAb11, mAb12, mAb13, mAb14, mAb15, mAb16, mAb17
	ND	mAb18, mAb19, mAb20

Table 1 The results of virus neutralization test in monoclonal antibodies
The monoclonal antibodies were isolated from COVID-19 patients. (n =20)

* The monoclonal antibody samples were kindly provided by Dr. Masaru Takeshita. (Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine)

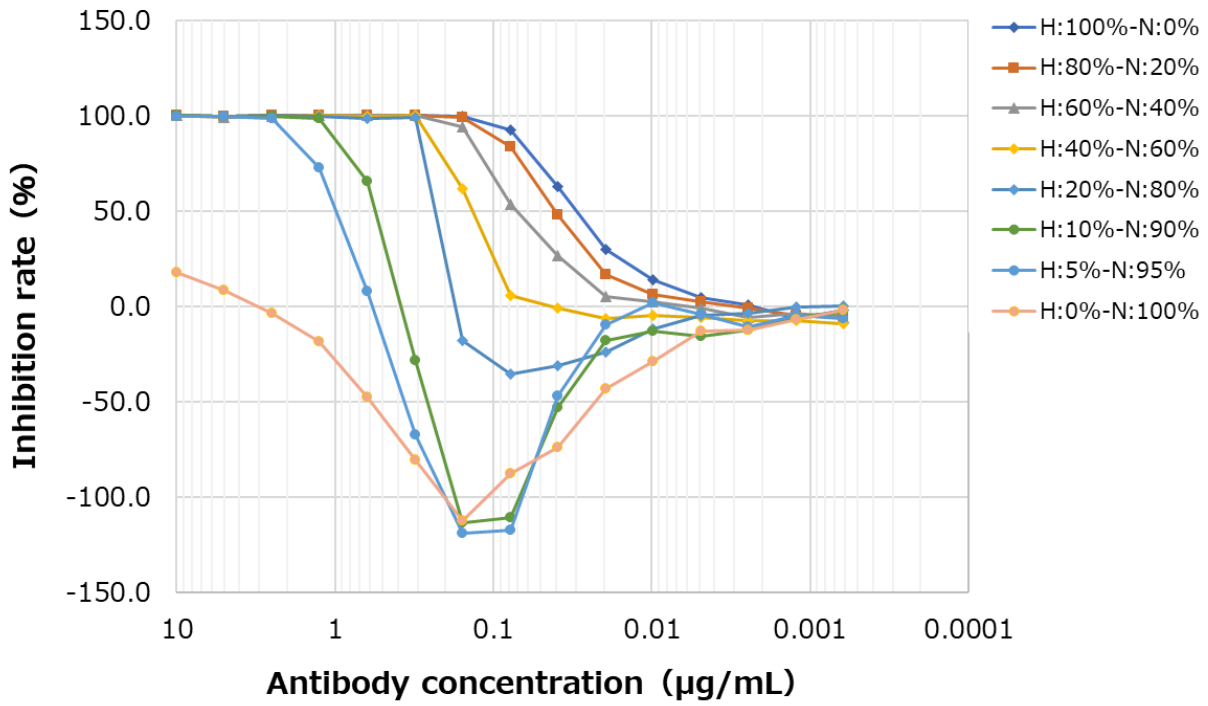


Fig.2 Inhibitory behavior of mixed monoclonal antibodies in the dilution series.
Inhibition behavior was evaluated by mixing two types of monoclonal antibodies with different neutralizing activities. Each of these antibodies was isolated from different individuals with COVID-19.
H; A monoclonal antibody with high neutralizing activity
N: A monoclonal antibody with non-neutralizing activity

* The monoclonal antibody samples were kindly provided by Dr. Masaru Takeshita. (Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine)

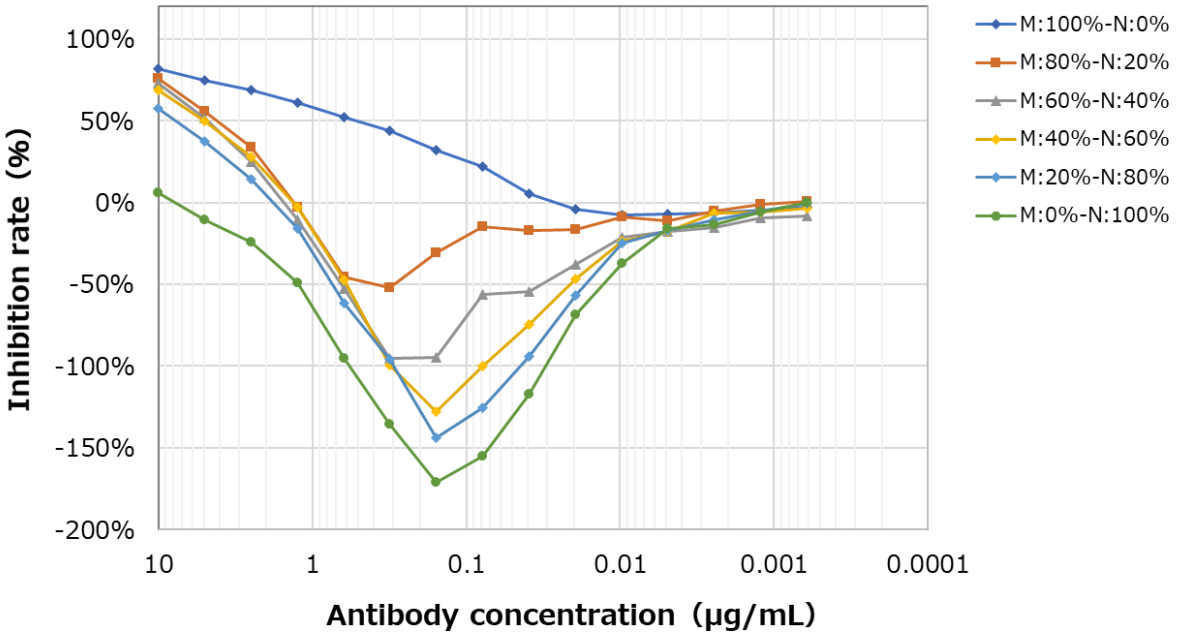


Fig.3 Inhibitory behavior of mixed monoclonal antibodies in the dilution series.
Inhibition behavior was evaluated by mixing two types of monoclonal antibodies with different neutralizing activities. Each of these antibodies was isolated from different individuals with COVID-19.
M; A monoclonal antibody with moderate neutralizing activity
N: A monoclonal antibody with non-neutralizing activity

* The monoclonal antibody samples were kindly provided by Dr. Masaru Takeshita. (Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine)

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