

Mouse Anti-SARS-CoV-2 Antibody IgG Titer Serologic Assay Kit

Pack Size: 96 tests

Catalog Number: RAS-T018

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedure

INTENDED USE

This kit is developed for semi-quantitative detection or titer measurement of Mouse Anti-SARS-CoV-2 Antibody IgG (Spike RBD) in mouse samples. It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

The ongoing SARS-COV-2 pandemic has sickened 3.7 million people around the world, claiming over 1,000,000 lives. To deal with this global public health crisis, unprecedented efforts are being made to study the virus, SARS-CoV-2. At present, many new vaccine projects registered with the World Health Organization are being developed. To support these studies, ACRO has developed Mouse Anti-SARS-CoV-2 antibody IgG Serologic Assay kit.

Principle of Mouse Anti-SARS-CoV-2 Antibody IgG Titer Serologic Assay kit is an indirect ELISA. The microplate in the kit has been pre-coated with SARS-CoV-2 Spike RBD. First add the diluted samples to the plate and wash the wells after incubation. Afterwards add HRP-Conjugated Antibody to the plate and wash the wells after incubation. Lastly the substrate is loaded into the wells and color develops in proportion to the amount of antibody. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm/630 nm. The OD Value reflects the amount of antibody bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAS018-C01	Pre-coated with SARS-CoV-2 Spike RBD Microplate	1 plate	Solid	2-8°C	2-8°C
RAS018-C02	Anti-SARS-CoV-2 Antibody (Control, Mouse IgG)	100 µL	Liquid	2-8°C	2-8°C
RAS018-C03	HRP-Conjugated Antibody	10 µg	Powder	2-8°C avoid light	-70°C avoid light
RAS018-C04	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS018-C05	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS018-C06	Substrate Solution	12 mL	Liquid	2-8°C avoid light	2-8°C avoid light
RAS018-C07	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

RAS018-C08	SARS-CoV-2 Antibody Positive Control	100 µL	Liquid	2-8°C	2-8°C
RAS018-C09	SARS-CoV-2 Antibody Negative Control	100 µL	Liquid	2-8°C	2-8°C

STORAGE AND VALIDITY INSTRUCTIONS

1. Unopened kit should be stored at 2°C-8°C upon receiving.
2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.
3. The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

MATERIALS REQUIRED BUT NOT PROVIDED

Dual wavelength microplate reader with 450 nm/630 nm filter;

Centrifuge;

37 °C Incubator;

Single channel or multichannel pipettes with 10 µL, 200 µL and 1000 µL precision;

10 µL, 200 µL and 1000 µL pipette tips;

Test Tubes;

Graduated cylinder;

Deionized or distilled water for dilution;

REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20°C-25°C) before use.
2. As recommended in Table 2, the lyophilized materials of HRP-Conjugated Antibody will be diluted into a rehydrated solution with ultrapure water/deionized water. Before use, the rehydrated solution needs to be balanced at room temperature of 30 min, shake gently every 10 min. Do not shake or vortex violently. The rehydrated solution should be stored at -70°C, Do not thaw and freeze more than 3 times. To avoid surface adsorption loss and inactivation, the reconstituted protein must NOT be aliquoted to less than 5 µg per vial.

Note: HRP-Anti-Human IgG stock solution should be protected from light.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

Catalog	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
RAS018-C03	HRP-Conjugated Antibody	10 µg	50 µg/mL	200 µL water

RECOMMENDED PROTOCOL

1. Working fluid preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of Positive Control and Negative Control working fluid and pre-treatment of samples:

a. For qualitative detection of antibodies:

Dilute the samples, Positive Control and Negative Control at 1:100 with Dilution Buffer.

b. For semi-quantitative detection or titer measurement of antibodies:

b.1 It is recommended dilute the samples from 1:100-1:12800 with Dilution Buffer.

b.2 If titer measurement, it is recommended dilute the Negative Control from 1:100-1:12800, and dilute the Positive Control from 1:100-1:51200 with Dilution Buffer.

If semi-quantitative detection, it is recommended dilute the Anti-SARS-CoV-2 Antibody (Control, Mouse IgG) from 0.2-6.25 ng/ml with Dilution Buffer. Please refer to the tube label for concentration.

2. Add Samples

Add 100 µL serially diluted samples, Positive Control, Negative Control or Anti-SARS-CoV-2 Antibody (Control, Mouse IgG) to each well. And for Blank Control wells, please add 100 µL Dilution Buffer to the well. Seal the plate with microplate sealing film and incubate at 37°C for 1.0 h. Avoid light.

3. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer: to each well, gently tap the plate for 30 s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels.

Repeat the wash step above for three times.

4. HRP-Anti-Human IgG

Dilute **HRP-Conjugated Antibody** stock solution (50 µg/mL) to 0.08 µg/mL with Dilution Buffer to make working solution. For all wells, add 100 µL **HRP-Conjugated Antibody** working solution, seal the plate with microplate sealing film and incubate at 37°C for 1.0 h, avoid light.

5. Washing

Repeat step 3.

6. Substrate Reaction

Add 100 μ L **Substrate Solution** to each well. Seal the plate with microplate sealing film and incubate at 37°C for 20 min, avoid light.

7. Termination

Add 50 μ L **Stop Solution** to each well, and tap the plate gently for 3 min to allow thorough mixing.

Note: the color in the wells should change from blue to yellow.

8. Data Recording

Read the absorbance at 450 nm/630 nm using UV/Vis microplate spectrophotometer.

Note: To reduce the background noise, subtract the value read at OD_{450 nm} with the value read at OD_{630 nm}.

CUT-OFF VALUE IDENTIFICATION

Cut-off value =0.1.

Note: The cut-off value can be determined by the end user.

Negative control at 1:100 dilution: OD_{450 nm}-OD_{630 nm} <0.1,

Positive control at 1:800 dilution:OD_{450 nm}-OD_{630 nm} ≥1.0

Note: If OD_{450 nm}-OD_{630 nm} values of controls do not meet the requirement, the test is invalid and must be repeated.

INTERPRETION OF RESULTS

Positive reading: OD_{450 nm}-OD_{630 nm} of sample ≥ Cut-off value means Anti-SARS-CoV-2 Antibody IgG (Spike RBD) are detected.

Negative reading: OD_{450 nm}-OD_{630 nm} of sample < Cut-off value means Anti-SARS-CoV-2 Antibody IgG (Spike RBD) are not detected.

a. For determination of antibody titer:

Determination of antibody titer: the positive sample was diluted with a gradient, and the antibody titer of the sample corresponds to the highest dilution factor that still yields a positive reading.

b. For semi-quantitative detection of antibodies:

If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated. To calibrate absorbance value obtained by the standard curve, the OD value of the sample to be measured is subtracted to the OD value of the blank control. The standard curve is plotted with the standard

concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic or other statistical software are used to draw the standard curve and calculate the sample concentration.

LIMITATIONS OF THE PROCEDURE

This test is designed for titer or semi quantitative detection of mouse Anti-SARS-CoV-2 Antibody IgG (Spike RBD). The kit cannot be used for quantitative detection.

LIMITATIONS OF THE PROCEDURE

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. This kit should be used according to the provided instructions.
3. Do not mix reagents from different lots.
4. Bring all reagents to room temperature (20°C-25°C) before use. If crystals have formed in the buffer solution, incubate until the crystals have completely dissolved. Before use, bring the solution back to room temperature.
5. This kit should be stored at 2°C-8°C.
6. Please prepare the working solution of each component according to the needs of the experiment, all prepared working solution is for one-time use and cannot be stored.