FT-BH4GV3

Anti-SARS-CoV-2 Neutralizing Antibody Titer Serologic Assay Kit

Catalog Number: TAS-K022 - Pack Size: 96 tests

Intended use

This kit is developed for detecting the titer of Anti-SARS-CoV-2 neutralizing antibody in serum in vitro. It is intended for research use only (RUO).

Principle Of The Assay

The newly identified Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is posing a serious threat to human health, and it spreads quickly. It is helpful to develop the Anti SARS-CoV-2 Neutralizing Antibody Titer Serologic Assay Kit to test the protective neutralizing antibody level in serum.

This assay kit is used to measure the levels of Anti-SARS-CoV-2 neutralizing antibody by employing a competitive ELISA. The microplate in the kit has been pre-coated with Human ACE2 protein. First add the samples and HRP- SARS-CoV-2 Spike RBD to the plate, incubate and wash the wells. Then load the substrate into the wells. The reaction is stopped by the addition of a stop solution and the intensity of the color can be measured at 450nm. The Anti-SARS- CoV-2 neutralizing antibody in the samples competes with immobilized Human ACE2 protein to combine with HRP- SARS-CoV-2 Spike RBD. The signal color becomes lighter as the content of Anti-SARS-CoV-2 neutralizing antibody.

Materials Provided

Table 1

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
TAS022-C01	Pre-coated with Human ACE2 Protein Microplate	1 plate	Solid	2-8°C	2-8°C
TAS022-C02	Anti-SARS-CoV-2 Neutralizing Antibody	10μg	Powder	2-8°C	-70°C
TAS022-C03	HRP-SARS-CoV-2 Spike RBD	10μg	Powder	2-8°C avoid light	-70°C, avoid light
TAS022-C04	10xWashing Buffer	50mL	Liquid	2-8°C	2-8°C
TAS022-C05	Dilution Buffer	50mL	Liquid	2-8°C	2-8°C
TAS022-C06	Substrate Solution	12mL	Liquid	2-8°C avoid light	2-8°C avoid light
TAS022-C07	Stop Solution	7mL	Liquid	2-8°C	2-8°C

Shipping and storage

This kit is shipped at room temperature.

The unopened kit is stable for at least 1 year from the date of manufacture if stored at 2°C to 8°C . The opened kit is stable for up to 1 months from the date of opening.

Reagent preparation

Reconstitute the provided lyophilized materials to stock solutions with distilled, sterile water as recommended in Table 2, Solubilize for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking or vertexing.

The reconstituted stock solutions should be stored at -70°C. **It is recommended not to freeze-thaw 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.

Table 2. Reconstitution methods for 96 tests

Catalog	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
TAS022-C02	Anti-SARS-CoV-2 Neutralizing Antibody	10μg	100μg/mL	100μL water
TAS022-C03	HRP-SARS-CoV-2 Spike RBD	10μg	100μg/mL	100μL water

AAll reagents should be balance to room temperature (20°C-25°C) before use. If crystals have formed in buffer solution, worm to room temperature until the crystals have completely dissolved.
1×Washing Buffer: prepare 500mL 1× Washing Buffer by adding 50mL 10 × Washing Buffer to 450mL distilled water..

Recommended protocol

1. Add Samples

Make series dilution of the tested samples with 1×Dilution Buffer. The recommended dilution of the sample is from 1:10 to 1:100. Dilute **HRP-SARS-CoV-2 Spike RBD** stock solution (100μg/mL) to 0.3μg/mL with 1×Dilution Buffer to make working solution. Add 50μL serially diluted samples and 50μL HRP-SARS-CoV-2 Spike RBD working solution to each well. And for negative control wells, please add 50μL HRP-SARS-CoV-2 Spike RBD working solution and 50μL Dilution Buffer to the well. Seal the plate with microplate sealing film and incubate at 37°C for 1 hour. Avoid light.

If the antibody concentration in the sample is analyzed semi-quantitatively, Anti-SARS-CoV-2 Neutralizing Antibody provided can be diluted with 1×Dilution Buffer, and the recommended concentration range of dilution is 5-160ng/mL.

2. Washing

Remove the remaining solution by aspiration, add 300μL 1 x Washing Buffer to each well, gently tap the plate for 1 minute, remove any remaining Washing Buffer by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

3. Substrate Reaction

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

4. Termination

Add 50μL Stop Solution to each well, and tap the plate gently for 3 minutes to allow thorough mixing.

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

5. Data Recording

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

Note: the plate may be read at 630nm and the signal-to-background ratio may be reduced.

Cut-off value identification

Cut-off value =20% signal inhibition.

Signal inhibition=(1-OD value of sample/ OD value of negative control)×100%

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

Interpretion of results

Positive: Signal inhibition of sample≥Cut-off value means Anti SARS-CoV-2 neutralizing antibody are detected.

Negative: Signal inhibition of sample<Cut-off value means Anti SARS-CoV-2 neutralizing antibody are not detected.

Calculation of igg titer

The maximum dilution multiple of the positive test results was selected, and the corresponding OD value of the maximum dilution / Cut-off × dilution multiple, the calculated value of was the antibody titer corresponding to the sample.

Limitations of the procedure

This test is designed for qualitative or semi quantitative detection of Anti SARS-CoV-2 neutralizing antibody.

Limitations of the procedure

1. This kit is for research use only and is not for use in diagnostic or therapeutic procedures.
2. The kit should be used according to the instructions.
3. Do not mix reagents from different lots.
4. All reagents should be balance to room temperature(20°C-25°C) before use. If crystals have formed in buffer solution, worm to room temperature until the crystals have completely dissolved.
5. The kit should be stored at 2°C to 8°C