FT-BH4GV2

SARS-CoVCoV-2 Neutralizing Antibody ELISA Kit)

Catalog Number: CSB-EL33243HU

For the qualitative determination of SARS-CoV-2 neutralizing antibody concentrations in serum, plasma.

Principle Of The Assay

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with RBD. Neutralizing Antibody or samples are added to the appropriate microtiter plate wells with Horseradish Peroxidase (HRP) conjugated ACE2. The competitive inhibition reaction is launched between with HRP-ACE2 and SARS-CoV-2 neutralizing antibody in samples. A substrate solution is added to the wells and the color develops in opposite to the amount of SARS-CoV-2 neutralizing antibody in the sample. The color development is stopped and the intensity of the color is measured.

Specificity

This assay has high sensitivity and excellent specificity for detection of SARSSARS-CoVCoV-2 neutralizing antibody.

Note: *Limited by current skills and knowledge, it is impossible for us to complete the crosscross-reactivity detection between SARS SARS-CoVCoV-2 neutralizin g antibody and all the analogues, therefore, cross reaction may still exist.*

Precision

Intra-assay Precision (Precision within an assay): CV% <15%

Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV% <15%

Three samples of known concentration were tested in twenty assays to assess.

Limitations of the procedure

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

Materials Provided

Catalog	Components
Reagents	Quantity
Assay plate (12 x 8 coated Microwells)	1(96 wells)
Neutralizing Antibody (10 x concentrate)	1 x 100 µL
HRP-conjugate (100 x concentrate)	1 x 60 µL
HRP-conjugate Diluent	1 x 10 mL
Sample Diluent	2 x 20 mL
Wash Buffer (25 x concentrate)	1 x 20 mL
TMB Substrate	1 x 10 mL
Stop Solution	1 x 10 mL
Adhesive Strip (For 96 wells)	4
Instruction manual	1

Storage

Unopened kit	Store at 2 - 8°C. Do not use the kit beyond the expiration date.
Opened kit	May be stored for up to one month at 2 - 8° C.

*Provided this is within the expiration date of the kit.

Other supplies required

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.

- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100mL and 500mL graduated cylinders.
- Deionized or distilled water.
- Pipettes and pip ette tips.
- Test tubes for dilution.

Precautions

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Sample collection and storage

- **Serum** Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thawcycles.
- **Plasma** Collect plasma using EDTA, or heparin as an anticoagulant.Centrifuge for 15 minutes at 1000 ×g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Sample preparation

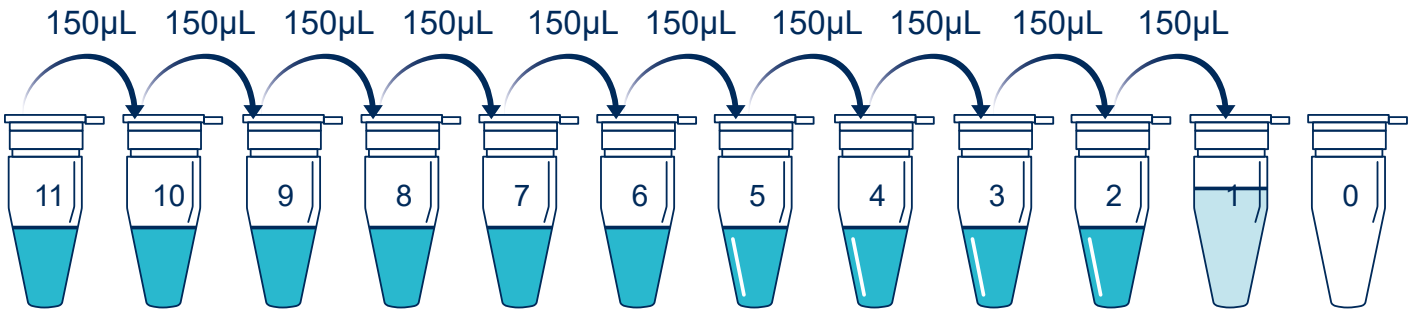
Recommend to dilute the serum or plasma samples with Sample Diluent (1:20) before test. The suggested 20-fold dilution can be achieved by adding 15μL sample to 285μL of Sample Diluent. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.

Note:

1. CUSABIO is only responsible for the kit itself, but not for the samplesconsumed during the assay. The user should calculate the possibleamount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 2 days may be stored at 2-8°C, otherwisesamples must be stored at -20°C (≤1month) or -80°C (≤2month) to avoid loss of bioactivity and contamination.
3. Grossly hemolyzed samples are not suitable for use in this assay.
4. If the samples are not indicated in the manual, a preliminary experiment todetermine the validity of the kit is necessary.
5. Fresh samples without long time storage are recommended for the test.Otherwise, protein degradation and denaturalization may occur in thosesamples and finally lead to wrong results.

1. **HRP-conjugate (1x)** - Centrifuge the vial before opening.
   HRP-conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10μL of **HRP-conjugate** + 990μL of **HRP-conjugate DiluentDiluent**.
2. **Wash Buffer (1x)**- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20mL of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500mL of Wash Buffer (1x).
3. **Neutralizing Antibody**
   Centrifuge the Neutralizing Antibody vial at 6000-10000rpm for 30s before opening.
   Dilute the **Neutralizing Antibody (10x)** with **Sample Diluent Diluent**. Asuggested 10-fold dilution is 30μL of **Neutralizing Antibody (10x)** + 270μL of **Sample Diluent**. This diluted Neutralizing Antibody (S11) serves as the high Neutralizing Antibody (10000ng/mL or 125 nM). Do not substitute other diluents. Mix the Neutralizing Antibody to ensurecomplete dilution and allow the Neutralizing Antibody to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

   Pipette 150μL of **Sample Diluent** into each tube (S0-S10). Use the diluted **Neutralizing Antibody** (S11) solution to produce a **2-fold** dilution series (below). Mix each tube thoroughly before the next transfer. **Sample Diluent** serves as the zero Neutralizing Antibody (0ng/mL or 0 nM).



Tube	S11	S10	S9	S8	S7	S6	S5	S4	S3	S2	S1	S0
ng/mL	10000	5000	2500	1250	625	312	156	78	39	19.5	9.75	0
nM	125	62.5	31.25	15.625	7.813	3.906	1.953	0.977	0.488	0.244	0.122	0

Assay procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and Ne utralizing Antibody be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a Blank well without any solution.
4. Add 50µL of **Neutralizing Antibody** and diluted **Sample** per well.
5. Add 50µL **HRP-conjugate (1x)** to each well immediately (not to Blank well). Mix well with the pipette or shake the plate gently for 60 seconds. A plate layout is provided to record Neutralizing Antibody and samples assayed.
6. Cover with the adhesive strip provided. Incubate for 1 hour at 37°C.
7. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with **Wash Buffer** (250µL) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
8. Add 90µL of **TMB Substrate** to each well. Incubate for 20 minutes at 37°C. **Protect from light**.
9. Add 50µL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450nm.

***Samples may require dilution. Please refer to Sample Preparation section.**

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or or reagents addition: Please carefully add samples to wells andmix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents orsamples to the assay plate should not exceed 10 minutes. This will ensureequal elapsed time for each pipetting step, without interruption. Duplication of all specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between sample additions, andbetween reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered forextended periods between incubation steps. Once reagents have beenadded to the well strips, DO NOT let the strips DRY at any time during theassay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop ofwater and fingerprint on the bottom of the plate. Insufficient washing willresult in poor precision and falsely elevated absorbance reading. Whenusing an automated plate washer, adding a 30 second soak period followingthe addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding TMBSubstrate (e.g. observation once every 10 minutes), TMB Substrate shouldchange from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. TMB Substrate is easily contaminated. TMB Substrate should remaincolorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the TMBSubstrate. The color developed in the wells will turn from blue to yellowupon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

Calculation of results

1. Average the duplicate readings for each Neutralizing Antibody and sample and subtract the average optical density of Blank.
2. Calculate the binding rate(%) rate(%) the OD value of Neutralizing Antibody and sample are divided by the OD value of S0 and multiplied by 100%.

$$\text{Binding rate (\%)} = \frac{B}{B_0} \times 100\%$$

B: the average absorbance value of the sample or Neutralizing Antibody

B_0: the average absorbance value of the 0ng/mL (0nM) Neutralizing Antibody

3. Draw the curve: take the Neutralizing Antibod Antibody concentration as the x x-axis and the corresponding percentage binding rate as the y y-axis, draw the smooth curve, and calculate the IC50, for reference.
4. Judgment cut-off = 1/2 OD OD_{S0}

   While ODsample < 1/2 OD_{S0}: Positive

   While ODsample ≥ 1/2 OD_{S0}: Negative