

Human Novel Coronavirus (SARS-Cov-2) Neutralizing Antibody ELISA Kit

Cat# E5019

Storage at 2-8°C

SPECIFICATION

Sensitivity: 9.75 ng/mL.**Detection Range:** 9.75 - 10000 ng/mL.

Specificity: This assay has high sensitivity and excellent specificity for detection of human SARS-CoV-2 neutralizing antibody. No significant cross-reactivity or interference between human SARS-CoV-2 neutralizing antibody and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between human SARS-CoV-2 neutralizing antibody and all the analogues, therefore, cross reaction may still exist

PRINCIPLE of KIT

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with RBD. Standards 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.

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.

CONTENTS and STORAGE

Reagents	Quantity
Assay plate (12 x 8 coated Microwells)	1(96 wells)
Standard (10 x concentrate)	1 x 200 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

Unopened kit	Store at 2 - 8°C. Do not use the kit beyond the expiration date.	
Opened kit	Coated assay plate	May be stored for up to 1 month at 2 - 8°C. Try to keep it in a sealed aluminum foil bag, and avoid the damp.
	Standard	May be stored for up to 1 month at 2 - 8° C. If don't make recent use, better keep it store at -20°C.
	HRP-conjugate	
	HRP-conjugate Diluent	May be stored for up to 1 month at 2 - 8°C.
	Sample Diluent	
	Wash Buffer	
	TMB Substrate	
	Stop Solution	

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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.
- If samples generate values higher than the highest standard, dilute the samples with Sample Diluent and repeat the assay.
- 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.

OTHER SUPPLIES REQUIRED

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
2. An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
3. Squirt bottle, manifold dispenser, or automated microplate washer.
4. Absorbent paper for blotting the microtiter plate.
5. 100ml and 500ml graduated cylinders.
6. Deionized or distilled water.
7. Pipettes and pipette tips.

8. 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

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-thaw cycles.

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. ACE Biolabs is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples 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 to determine the validity of the kit is necessary.
5. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
6. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
7. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
8. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENT PREPARATION

Note:

- Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
- Bring all reagents to room temperature (18-25°C) before use for 30min.
- Prepare fresh standard for each assay. Use within 4 hours and discard after use.
- Making serial dilution in the wells directly is not permitted.
- Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

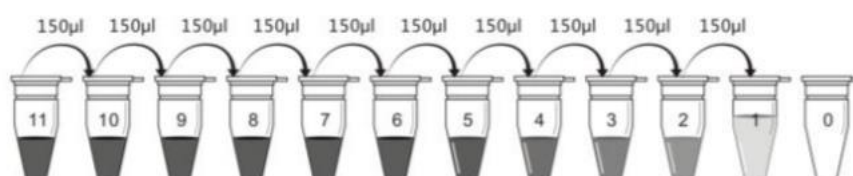
1. HRP-conjugate (1x) - Centrifuge the vial before opening.

HRP-conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of HRP-conjugate + 990 µl of HRP-conjugate Diluent.

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 20 ml of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).

3. Standard

Centrifuge the standard vial at 6000-10000rpm for 30s before opening. Dilute the Standard(10x) with Sample Diluent. A suggested 10-fold dilution is 30 µl of Standard(10x) + 270 µl of Sample Diluent. This diluted Standard (S11) serves as the high standard (10000 ng/ml). Do not substitute other diluents. Mix the standard to ensure complete dilution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 150 µl of Sample Diluent into each tube (S0-S10). Use the diluted Standard (S11) solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. Sample Diluent serves as the zero standard (0 ng/ml).



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

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 standards be assayed in duplicate.

1. Prepare all reagents, working standards, 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 standard and sample per well. 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 standards and samples assayed.
5. Cover with the adhesive strip provided. Incubate for 1 hour at 37°C.
6. 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.
7. Add 90µl of TMB Substrate to each well. Incubate for 20 minutes at 37°C. Protect from light.
8. Add 50µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

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

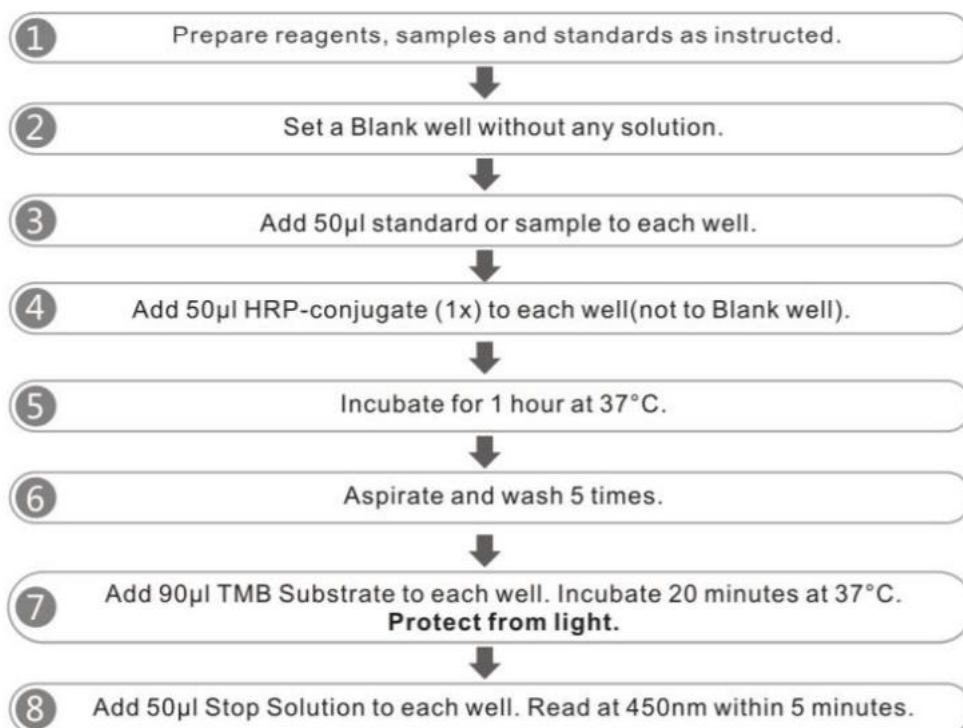
NOTE

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 reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix 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 or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between 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 for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. 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 of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the 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 TMB Substrate (e.g. observation once

every 10 minutes), TMB Substrate should change 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 remain colorless 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 TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

ASSAY PROCEDURE SUMMARY



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

CALCULATION of RESULTS

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

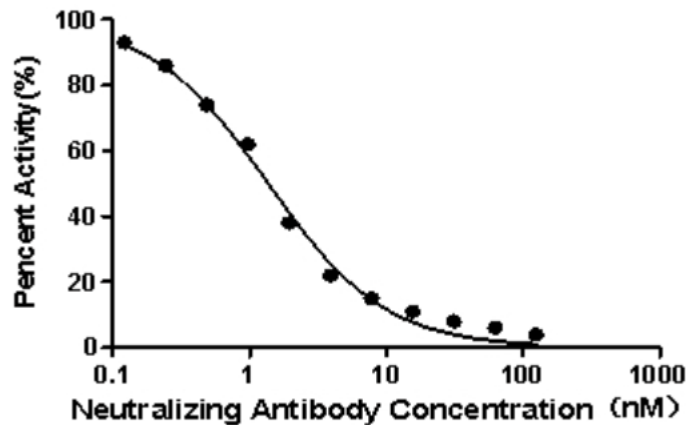
$$\text{Binding rate (\%)} = \frac{B}{B_0} \times 100\%$$

B —the average absorbance value of the sample or standard

B₀ —the average absorbance value of the 0 ng/ml standard

3. Draw the curve: take the standard concentration as the x-axis and the corresponding percentage binding rate as the y-axis, draw the smooth curve, and calculate the IC₅₀, for reference.
4. Judgment : cut-off = 1/2 OD₅₀
 - While OD_{sample} < 1/2 OD₅₀: Positive
 - While OD_{sample} ≥ 1/2 OD₅₀: Negative

TYPICAL DATA



g/ml	nM	OD1	OD2	Average	percent activity(%)
10000	125	0.099	0.092	0.096	4
5000	62.5	0.145	0.136	0.141	6
2500	31.25	0.194	0.183	0.189	8
1250	15.625	0.274	0.263	0.269	11
625	7.813	0.344	0.371	0.358	15
312	3.906	0.519	0.528	0.524	22
156	1.953	0.901	0.907	0.904	38
78	0.977	1.509	1.412	1.461	62
39	0.488	1.787	1.723	1.755	74
19.5	0.244	2.121	1.958	2.040	86
9.75	0.122	2.233	2.138	2.186	93
0	0	2.394	2.328	2.361	

The inhibition curve of SARS-CoV-2 Inhibitor to the binding between RBD and ACE2, IC₅₀=1.376 nM