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COVID-19 Spike Protein ELISA Kit

48T/96 Tests

Catalogue Number:SL5066Hu

Store all reagents at 2-8℃

Validity Period: six months

For samples:

In human serum, plasma, saliva and nasal fluid.

FOR RESEARCH USE ONLY !

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS !

PLEASE READTHROUGH ENTIRE PROCEDURE BEFORE BEGINNING !

COVID-19 Spike Protein ELISA Kit

FOR RESEARCH USE ONLY

Reactivity: Coronavirus(COVID-19,SARS-CoV-2, 2019-nCoV, Novel Coronavirus Spike Glycoprotein,RBD, SARS-CoV-2 S1)

Range: 0.78-50pg/ml

Sensitivity: 390 **f**g/ml

Application: For quantitative detection of 2019 nCoV(S) in serum, plasma, tissue homogenates, cell culture supernatant, cell culture lysate and other biological fluids(throat swab, nose swab)

Storage: 2-8°C for 6 months

Principle: Indirect

NOTE: FOR RESEARCH USE ONLY.

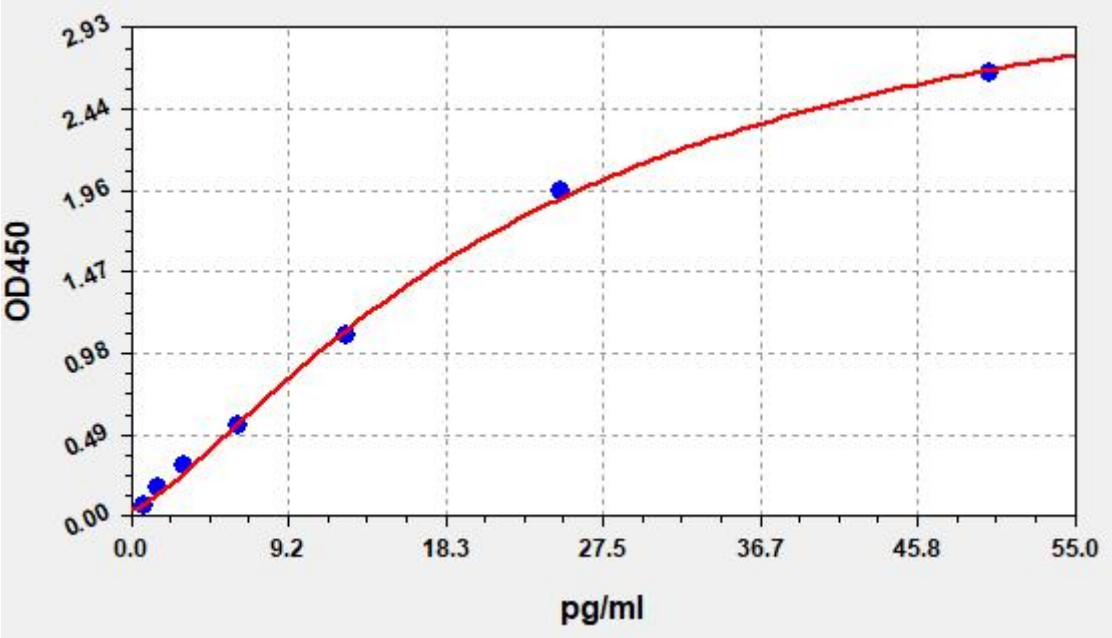
Kit Components

Item	**Specifications(48T/96T)**	**Storage**
ELISA Microplate(Dismountable)	8×6/8×12	2-8°C/-20°C
Lyophilized Standard	1vial/2vial	2-8°C/-20°C
Sample/Standard Dilution Buffer	5ml/10ml	2-8°C
Biotin-labeled Antibody(Concentrated)	60ul/120ul	2-8°C(Avoid Direct Light)
Assay Dilution	2.5ml/5ml	2-8°C
Antibody Dilution Buffer	5ml/10ml	2-8°C
HRP-Streptavidin Conjugate(SABC)	60ul/120ul	2-8°C(Avoid Direct Light)
SABC Dilution Buffer	5ml/10ml	2-8°C
TMB Substrate A	2.5ml/5ml	2-8°C(Avoid Direct Light)
TMB Substrate B	2.5ml/5ml	2-8°C(Avoid Direct Light)
Stop Solution	5ml/10ml	2-8°C
Wash Buffer(25X)	15ml/30ml	2-8°C
Plate Sealer	3/5pieces	
Product Description	1copy	

Typical Data & Standard Curve

Results of a typical standard operation of a 2019 nCoV(S) ELISA Kit are listed below. This standard curve was generated at our lab for demonstration purpose only. Users shall obtain standard curve as per experiment by themselves. (N/A=not applicable)

STD.(pg/ml)	OD-1	OD-2	Average	Corrected
0	0.077	0.079	0.078	0.000
0.78	0.145	0.153	0.149	0.071
1.56	0.246	0.258	0.252	0.174
3.13	0.397	0.379	0.388	0.310
6.25	0.610	0.628	0.619	0.541
12.5	1.131	1.189	1.16	1.082
25	2.054	2.004	2.029	1.951
50	2.677	2.811	2.744	2.666



Sample test results

293T cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate, Cells were transfected with the 2019 nCoV(S) plasmid, Aliquots of the cell culture supernates were removed and assayed for levels of 2019 nCoV(S).

Condition/Culture times	After 2 hours	After 1 day
transfected	20pg/ml	300pg/ml
untransfected	ND	ND

Specificity

type of virus	cross reaction	type of virus	cross reaction
SARS-CoV-2	100%	MERS	20%
SARS	68%	H1N1(A/B)	< 2%

Recovery

Matrices listed below were spiked with certain level of 2019 nCoV(S) and the recovery rates were calculated by comparing the measured value to the expected amount of 2019 nCoV(S) in samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	87-105	95
EDTA Plasma(n=5)	90-103	97
Heparin Plasma(n=5)	86-104	97
Saliva(n=5)	85-102	93

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of 2019 nCoV(S) and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8
Serum(n=5)	88-102%	87-105%	91-103%
EDTA Plasma(n=5)	86-101%	83-101%	82-98%
Heparin Plasma(n=5)	81-98%	83-97%	90-96%
Saliva(n=5)	80-92%	90-102%	91-106%

Precision

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage condition.

Standard(n=5)	37°C for 1 month	2-8°C for 6 months

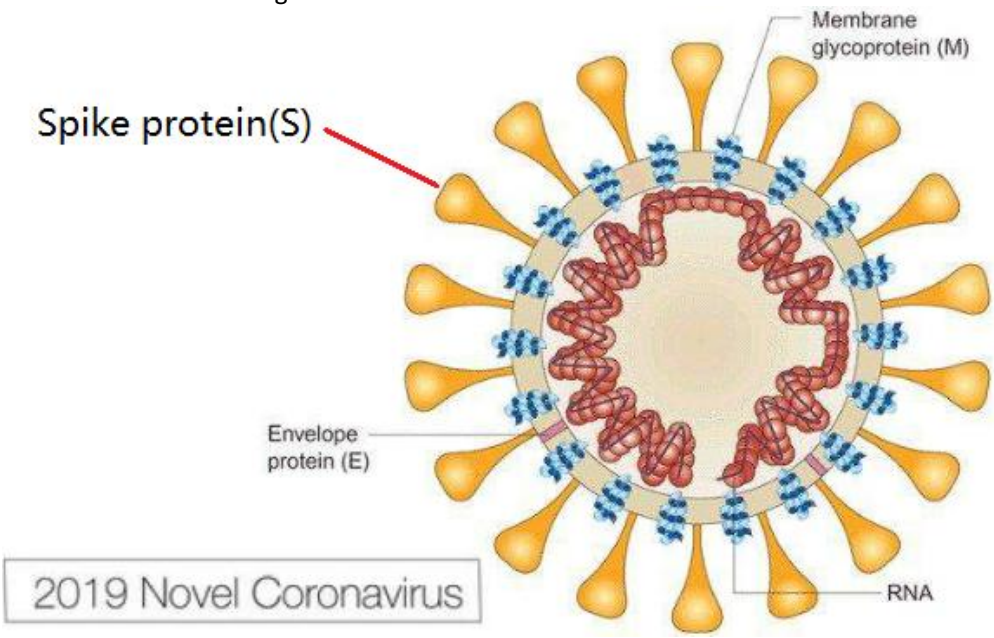
Average (%)	80	95-100

To minimize extra influence on performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is strongly suggested that the same operator performs the whole assay from the beginning to the end.

Operation Procedure

Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Capture antibody was pre-coated onto 96-well plates. And the biotin conjugated antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of target can be calculated.



Immunogen

Recombinant fragment within 2019 nCoV(S) aa 13 to 1273;

UniProt accession: P0DTC2

Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don’t let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don’t reuse tips and tubes to avoid cross contamination.
9. Avoid using the reagents from different batches together.

Material Required but Not Supplied

1. Microplate reader (wavelength:450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Washing

Manual: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.

Automatic: Aspirate all wells, and then wash plate with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer shall be set for soaking 1 minute. (**Note:** set the height of the needles; be sure the fluid can be sipped up completely)

Sample Collection and Storage (universal)

- **Serum:** Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and centrifugation for 20 minutes at approximately 1000×g, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
- **Plasma:** Collect plasma using (EDTA-Na_2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- **Other Biological Fluids:** Centrifuge samples for 20 minutes at 1000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

Note: Samples to be used within 5 days can be stored at 2-8°C, besides that, samples must be stored at -20°C (assay ≤1 month) or -80°C(assay≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for this assay.

Sample Dilution

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with Sample Dilution.

The matrix components in the sample will affect the test results, so it need to be diluted at least 1/2 with Sample Dilution Buffer before testing!

Reagent Preparation and Storage

Bring all reagents and samples to room temperature for 20 minutes before use.

1, Wash Buffer:

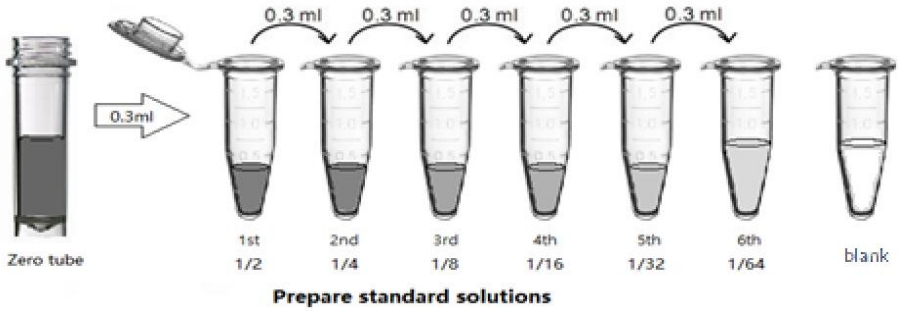
If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.

Dilute 30ml Concentrated Wash Buffer into 750ml Wash Buffer with deionized or distilled water. Put unused solution back at 2-8°C.

2, Standards:

1). Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the Sample Dilution Buffer into each tube. Add 0.3ml of the Standard solution (from zero tube) into 1st tube and mix them thoroughly. Transfer 0.3ml from

1st tube to 2nd tube and mix them thoroughly. Transfer 0.3ml from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample Dilution Buffer was used for the blank control.



Prepare standard solutions

Note: It is best to use Standard Solutions within 15 minutes.

3, Preparation of Biotin-labeled Antibody Working Solution:

Prepare it within 1 hour before experiment.

1）Calculate required total volume of the working solution: 0.1ml/well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)

2）Dilute the Biotin-detection antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul Biotin-labeled antibody into 99ul Antibody Dilution Buffer.)

4, Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:

Prepare it within 30 minutes before experiment.

1）Calculate required total volume of the working solution: 0.1ml/well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)

2）Dilute the SABC with SABC Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul of SABC into 99ul of SABC Dilution Buffer.)

5, Preparation of TMB Substrate Working Solution:

1）Calculate required total volume of the working solution: 0.09ml/well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)

2）Substrate A and B should be mixed together in equal volumes within 15 minutes of use. Avoid Direct Light. 90 μL of the resultant mixture is required per well.（note: Use different tips to soak Substrate A and B to avoid cross contamination）

Assay Procedure

When diluting samples and reagents, they must be mixed completely and evenly. It is recommended to plot a standard curve for each test.

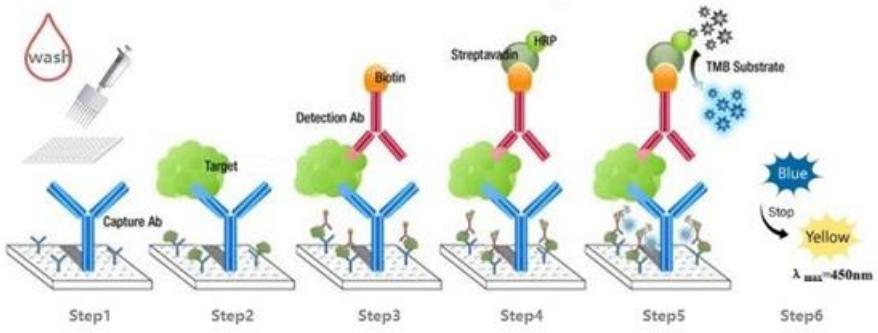
1. Set standard, test samples, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate.
2. **Wash plate 2 times** and then add 50μL of Assay Diluent to each well.
3. **Prepare Standards:** Aliquot 50ul of zero tube, 1sttube, 2ndtube, 3rdtube, 4thtube, 5thtube, 6thtube and Sample Dilution Buffer (blank) into the standard wells.
4. **Incubate:** Seal the plate with a cover and incubate at 37°C for 90 minutes.
5. **Wash:** Remove the cover and discard the plate content, and wash plate 2 times with Wash Buffer. Do NOT let the wells dry completely at any time.
6. **Biotin-labeled Antibody:** Add 100ul Biotin-labeled antibody working solution into above wells (standard, test sample and blank wells). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 60 minutes.
7. **Wash:** Remove the cover, and wash plate 3 times with Wash Buffer, and let the Wash Buffer stay in the wells for 1-2 minutes each time.

8. **HRP-Streptavidin Conjugate (SABC):** Add 100ul of SABC Working Solution into each well, cover the plate and incubate at 37°C for 30 minutes.
9. **Wash:** Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minutes each time.
10. **TMB Substrate Working Solution:** Add 90ul TMB Substrate Working Solution into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. (**Note:** The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)
11. **Stop:** Add 50ul Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
12. **OD Measurement:** Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the stop solution.

Regarding calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation, such as **Curve Expert 1.3 or 1.4.**

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary



Step1: Wash plate 2 times and then add 50µL of Assay Diluent to each well

Step2: Add 50ul standard or sample to each well and incubate for 90 minutes at 37°C

Wash step: Aspirate and wash plates 2 times.

Step3: Add 100ul Biotin-labeled antibody working solution to each well and incubate for 60 minutes at 37°C.

Wash step: Aspirate and wash plates 3 times.

Step4: Add 100ul SABC Working Solution into each well and incubate for 30 minutes at 37°C.

Wash step: Aspirate and wash plates 5 times.

Step5: Add 90ul TMB Substrate working Solution. Incubate 10-20 minutes at 37°C.

Step6: Add 50ul Stop Solution. Read at 450nm immediately and calculation.