For Research Use Only. Not for use in Diagnostic Procedures.

SARS-CoV-2 (S) IgG ELISA Kit (SCSE-IgG)

Catalog Number: EL2042-01

1. Application

This immunoassay kit allows for the qualitative determination of anti-nCoV-IgG in human serum, plasma, saliva and nasal fluid.

- **Size:** 96T
- **Reactivity:** Human

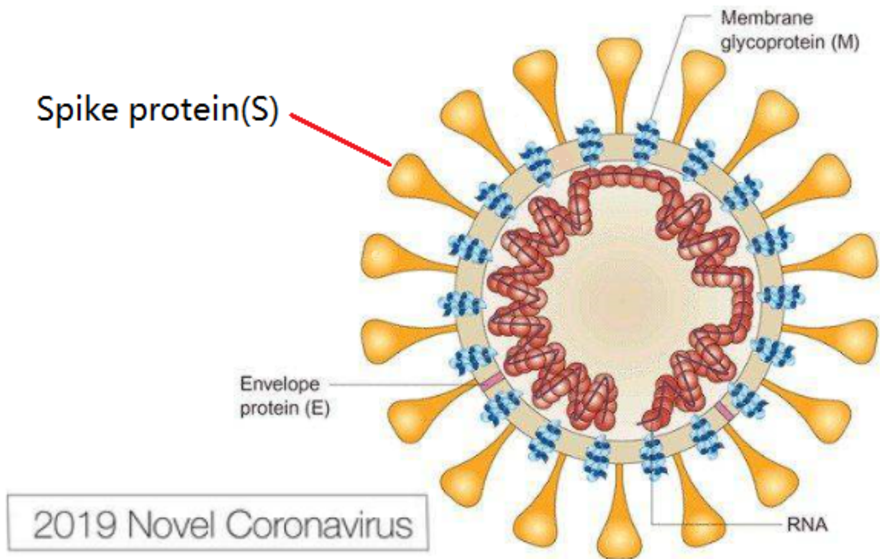
2. Storage and Kit Contents

Storage: 2-8℃ for 6 months.

Kit Components	Specifications (96T)	Storage
ELISA Microplate (Dismountable)	8×12	2-8°C
Negative Control (Ready-to-use)	1 vial	2-8°C
Positive Control (Ready-to-use)	1 vial	2-8°C
Sample Dilution Buffer	1 vial	2-8°C
HRP-conjugated anti-human IgG antibody (Concentrated)	1 vial	2-8°C
Antibody Dilution Buffer	1 vial	2-8°C
Wash Buffer (25 x concentrate)	1 vial	2-8°C
TMB Substrate	1 vial	2-8°C
Stop solution	1 vial	2-8°C
Plate Sealer	3 each	
Instruction manual	1 copy	

3. Principle of the Assay

This kit was based on indirect enzyme-linked immune-sorbent assay technology. Recombinant nCoV Spike protein (antigen) was pre-coated onto 96-well plates. The test samples were added to the wells, unbound conjugates were washed away with wash buffer. Then added HRP conjugated anti-human IgG, if there were any anti-nCoV-IgG in the samples, it would form a complex. TMB substrates were used to visualize HRP enzymatic reaction. It was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.



4. Sequence of spike protein RBD (antigen)

RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGV

SPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKV

GGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPY

RVVVLSFELLHAPATVCGPKKSTNLVKNKCVNF

5. Precautions

1. After opening and before using, keep plate dry.
2. Before using the Kit, balance the reagents at room temperature at least 30 mins.
3. Storage TMB reagents avoid light.
4. Washing process is very important, not fully wash easily cause a false positive.
5. Don't let Micro plate dry at the assay, for dry plate will inactivate active components on plate.
6. Don't reuse tips and tubes to avoid cross contamination.
7. Avoid using the reagents from different batches together.

6. User Supplied Materials

- Microplate reader (wavelength: 450nm)
- 37℃ incubator
- Automated plate washer
- Precision single and multi-channel pipette and disposable tips
- Clean tubes and Eppendorf tubes
- Deionized or distilled water

7. 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 350μl 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 350μl 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)

8. Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20℃ for long term. Avoid multiple freeze-thaw cycles.

- **Serum:** Coagulate the serum at room temperature (about 1 hour). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20℃.
- **Plasma:** Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 x g. Analyze immediately or aliquot and store frozen at -20℃.
- **Saliva & Nasal fluid:** Centrifuge samples for 20 minutes at 10000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

Note: Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20℃ (≤1 month) or -80℃(≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

9. Wash Buffer Preparation

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.

10. Preparation of HRP-conjugated anti-human IgG Working Solution

Prepare it within 1 hour before experiment.

1. Calculate required total volume of the working solution: 50μl / well × quantity of wells. (Allow 55-60μl more than the total volume.)
2. Dilute the HRP-conjugated anti-human IgG with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1μl HRP-conjugated anti-human IgG into 99μl Antibody Dilution Buffer.)

11. Assay Procedure

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 min at 37 °C. It is recommended to plot a standard curve for each test.

1. Bring all reagents to room temperature before use.
2. Label the sample wells, 2 Negative Controls, 2 Positive Controls and 1 blank well.

   Wash plate 2 times before adding sample and control (blank) wells!

3. Add 49 μL sample dilution buffer to each sample well.

   Add 50 μL sample dilution buffer for blank well.

4. Add 1μL sample to each sample well.

   Add 50ul Negative Controls and Positive Controls to set Controls well and gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37℃ for 30 min.

5. Remove the cover, and wash plate 3 times with Wash buffer and each time let the wash buffer stay in the wells for 1-2 min.

6. Add 50 μL HRP-conjugated anti-human IgG Working Solution to each well, except blank well.

7. Seal the plate with a cover and incubate at 37℃ for 30 min.

8. Remove the cover, and wash plate 5 times with Wash buffer and each time let the wash buffer stay in the wells for 1-2 min.

9. Add 50 μl of TMB substrate into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37℃ in dark within 10 min. And the shades of obvious blue can be seen in the Positive Controls. Blank well wells show no obvious color.

10. Add 50 μl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.

11. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution (Use the blank well to set zero).

12. Data Analysis

Calculation of Results

Cutoff Value =NCx × 2.1

NCx: Mean Absorbance of Negative Control (when NCx<0.05, Calculate as 0.05).

PCx : Mean Absorbance of Positive Control

1. Sample with absorbance values < Cutoff Value are considered negative.
   Sample with absorbance value ≥ Cutoff Value are considered positive.
2. PCx≤0.5, the test is regarded as Invalid, should be tested again.

13. Sample test data (For Reference Only)

Samples came from rehabilitation clients of mobile cabin hospital. The plasma samples were diluted 1:50. TMB Color development time was 10 minutes at 37℃. NCx= 0.086

Rehabilitation clients (OD450)				Healthy volunteers (OD450)			
1#	1.272	1#	1.542	1#	0.078	1#	0.092
2#	0.095	2#	0.096	2#	0.066	2#	0.095
3#	1.433	3#	1.556	3#	0.093	3#	0.087
4#	1.251	4#	1.372	4#	0.102	4#	0.088
5#	1.016	5#	1.004	5#	0.096	5#	0.079
6#	1.107	6#	1.106	6#	0.084	6#	0.085
7#	1.244	7#	1.223	7#	0.090	7#	0.076
8#	0.087	8#	0.065	8#	0.082	blank	0.058

Who we are

Attogene is a biotechnology company located in Austin, Texas. Our focus is to enhance health and wellness by offering and developing customer focused Life Science Products domestically and internationally.

Our mission is to:

- Enhance detection technologies
- Enable rapid responses
- Enable impactful research discoveries

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