Creative Diagnostics®

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User's Manual

SARS-CoV-2 S1 IgG Titer ELISA Kit

REF **DEIASL193**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This kit providing a rapid detection of anti-SARS-CoV-2 IgG in serum by SARS-CoV-2 Spike protein S1.

General Description

The newly identified Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has posed a serious threat to human health. A rapid and effective assay kit detecting the levels of anti-SARS-CoV-2 in human serum can facilitate research on characterization of antibodies produced in response to SARS-CoV-2 infection.

Principles of Testing

This assay kit employs a standard indirect-ELISA format, providing a rapid detection of anti-SARS-CoV-2 IgG in serum by SARS-CoV-2 Spike protein S1. The kit consists of High-bind Plate, Spike protein S1, an Anti-SARS-CoV-2 Antibody (Control, IgG), an HRP-Anti-Human IgG secondary antibody and Blocking/Dilution buffer.

Your experiment will include 6 simple steps:

a) Coat the plate with SARS-CoV-2 Spike protein S1.

b) Wash the plate with universal ELISA buffer and block the plate with the blocking buffer.

c) Add your sample to the plate, take the Anti-SARS-CoV-2 antibody as Control sample. The samples and Control sample are diluted by Blocking / Dilution Buffer.

d) Add a diluted Secondary antibody HRP-Anti-Human IgG to the plate. The Secondary antibody is diluted by Blocking / Dilution Buffer.

e) Wash the plate and add TMB or other colorimetric HRP substrate.

f) Stop the substrate reaction by add diluted acid. Absorbance (OD) is calculate as the absorbance at 450 nm minus the absorbance at 630 nm to remove background prior to statistical analysis. The OD Value reflects the amount of antibody bound.

Reagents And Materials Provided

1. High-bind Plate: 96 tests, solid
2. SARS-CoV-2 Spike protein S1: 30 μg, powder
3. Anti-SARS-CoV-2 Antibody (Control, IgG): 10 μg, Powder
4. HRP-Anti-Human IgG: 10 μg, Powder
5. Blocking / Dilution Buffer: 60 mL

Materials Required But Not Supplied

Coating Buffer 15 mmol/L sodium carbonate (Na_2CO_3), 35 mmol/L sodium hydrogen carbonate ($NaHCO_3$), pH 9.6, 12 mL is sufficient for 96 tests.

Wash Buffer PBS with 0.05% (v/v) Tween-20 (pH7.4), 500 mL is sufficient for 96 tests.

Substrate Dilution Buffer 50 mM disodium hydrogen phosphate (Na_2HPO_4) and 25 mM citric acid, adjust pH to 5.5 with 1 M Sodium hydroxide (NaOH), 25 mL is sufficient for 96 tests.

Substrate Stock Solution 20 mg/mL TMB (Sigma-Aldrich, Catalog # 860336) in Dimethyl sulfoxide (Sigma-Aldrich, Catalog # D8418), 1 mL is sufficient for 96 tests. Protect from light.

TMB Substrate Working Solution For each plate dilute 125 µL substrate stock solution in 25 mL substrate dilution buffer and add 20 µL 3% H_2O_2 (pipette 10 µL 30% H_2O_2 into 90 µL distilled water), mix well.

Notes:

1) The TMB Substrate Working Solution should be freshly prepared and used within 15 minutes.
2) If you choose to use other commercially available ready-to-use TMB substrate solutions, you should follow the manufacturer's instruction.

Stop Solution 1 M sulfuric acid (aqueous), 6 mL is sufficient for 96 tests.

Pipettes and pipette tips

UV/Vis microplate spectrophotometer (absorbance 450 nm, correction wavelength set to 630 nm).

Storage

This product is stable after storage at:

12 months in sealed state;

-20°C for 1 year in lyophilized state.

Reconstitution And Storage

Reconstitute the provided lyophilized materials to stock solutions with water as recommended in the following table, Solubilize for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking or vortexing.

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.

Note: HRP-Anti-Human IgG stock solution should be protected from light.

Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
High-bind Plate	96 tests	NA	NA
SARS-CoV-2 Spike protein S1	30 µg	200 µg/mL	150 µL water
Anti-SARS-CoV-2 Antibody (Control, IgG)	10 µg	100 µg/mL	100 µL water
HRP-Anti-Human IgG	10 µg	100 µg/mL	100 µL water
Blocking / Dilution Buffer	60 mL	NA	NA

All components are shipped at room temperature.

Upon receipt, please store the lyophilized products at -20°C to -70°C. After reconstitution, the stock solution should be kept at -70°C.

Upon receipt, please store the plate at Room Temperature, and please store the buffer at -20°C.

It is recommended not to freeze thaw more than 3 times.

Assay Procedure

1. Preparation

Reconstitute lyophilized reagents and store all reagents as recommended.

2. Coating

1) Dilute SARS-CoV-2 Spike protein S1 stock solution (200 μg/mL) to 2 μg/mL with Coating Buffer to make SARS-CoV-2 Spike protein S1 working solution.
2) Please leave two wells uncoated for No-Coating Control (Table for assay protocol).
3) Add 100 μL of SARS-CoV-2 Spike protein S1 working solution (2 μg/mL) to each well, seal the plate with microplate sealing film and incubate overnight (or 16 hours) at 4°C.

3. Washing

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

4. Blocking

Add 100 μL Blocking Buffer provided to each well, seal the plate with microplate sealing film and incubate at 37°C for 1.5 hours.

5. Washing

Repeat step 3. At meantime, you can start to prepare your samples.

6. Add Samples

1) Make series dilution of the Anti-SARS-CoV-2 Antibody (Control, IgG) as a reference (Ref.) with Dilution Buffer with 1:50 serum.
2) Make series dilution of the samples as recommended in Figure 1. And plate layout as recommended in Figure 2, the dilution buffer is Dilution Buffer with 1:50 serum.
3) For No-Binding Control wells, please add 100 μL Dilution Buffer with 1:50 serum.
4) For No-Coating Control wells, please add 100 μL Dilution Buffer with 1:50 serum.
5) For all other wells, Add 100 μL of sample solution to each well according to our recommendation (Figure 2) or your own plate setup.
6) seal the plate with microplate sealing film and incubate at 37°C for 1 hour, avoid light.

FIGURE 1. PREPARATION OF 1:2 SERIAL DILUTIONS OF THE ANTI-SARS-COV-2 ANTIBODY

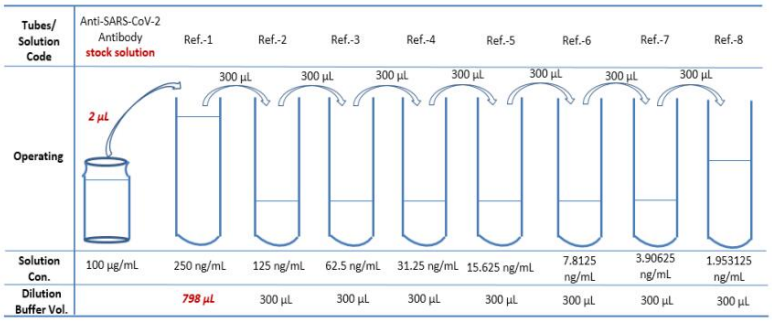
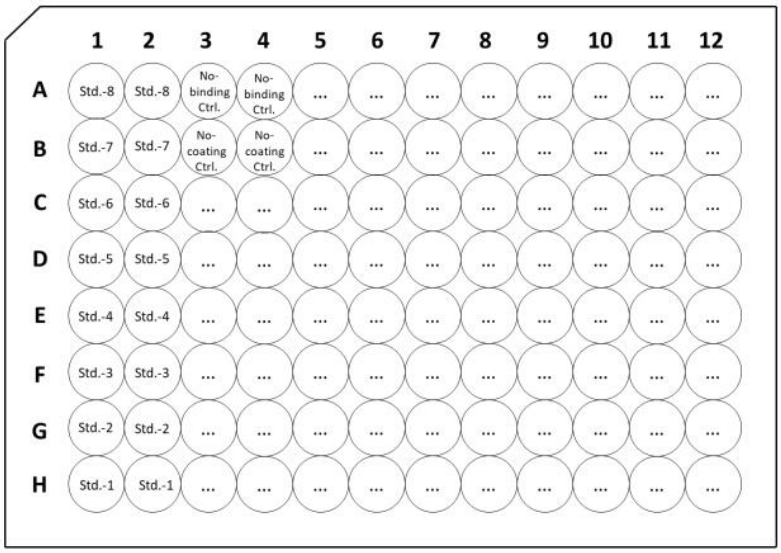


FIGURE 2. PLATE LAYOUT



7. Washing

Repeat step 3.

8. HRP-Anti-Human IgG

1) Dilute HRP-Anti-Human IgG stock solution (100 µg/mL) to 0.04 µg/mL with Dilution Buffer to make HRP-AntiHuman IgG working solution.

2) For all wells, add 100 µL HRP-Anti-Human IgG working solution, seal the plate with microplate sealing film and incubate at 37°C for 1 hour, avoid light.

9. Washing

Repeat step 3.

10. TMB Substrate Reaction

Add 200 µL TMB Substrate Working Solution to each well. Seal the plate with microplate sealing film and incubate at 37°C for 20 minutes, avoid light.

11. Termination

Add 50 µL 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.

12. Data Recording

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

Note: the plate may be read at 600 nm without adding 1 M sulfuric acid, but the Signal-to-Background ratio may be reduced.

Table for assay protocol:

Steps Code	Steps	Reagents & Instruments	Reaction Conditions	Samples	No-binding Control	No-coating Control
1	Preparation	N/A	N/A	N/A	N/A	N/A
2	Coating	SARS-CoV-2 Spike protein S1	4℃ for overnight	100 µL	100 µL	—
3	Washing	Wash Buffer	Wash for 3 times	300 µL	300 µL	300 µL
4	Blocking	Blocking Buffer	37℃ for 1.5 hours	100 µL	100 µL	100 µL
5	Washing	Wash Buffer	Wash for 3 times	300 µL	300 µL	300 µL
6	Add Samples	Samples	Incubate at 37℃ for 1 hour	100 µL	—	—
		Dilution Buffer		—	100 µL	100 µL
7	Washing	Wash Buffer 2	Wash for 3 times	300 µL	300 µL	300 µL
8	HRP-Anti-Human IgG	HRP-Anti-Human IgG Working Solution	37℃ for 1 hours	100 µL	100 µL	100 µL
9	Washing	Wash Buffer 2	Wash for 3 times	300 µL	300 µL	300 µL
10	TMB Substrate Reaction	TMB Substrate Working Solution	37℃ for 20 minutes	200 µL	200 µL	200 µL
11	Stop the Reaction	Stop Solution	Mix by gentle tapping for 3 minutes	50 µL	50 µL	50 µL
12	Data Recording	UV/Vis spectrophotometer	Measure absorbance at 450 nm, with the correction wavelength set at 600 nm			

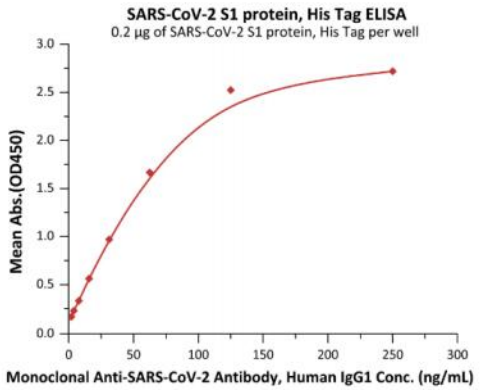
Note for table :

1) Samples: Your samples of interest.
2) No-Binding Control: Reaction without samples added. The absorbance should be around 0.05(< 0.1) at 450 nm.
3) No-Coating Control: Reaction without SARS-CoV-2 Spike protein S1 coated on the wells. The absorbance should be around 0.05(< 0.1) at 450 nm.
4) It is recommended that all samples, controls and references should be done in duplicates.

Calculation

Detection of Monoclonal Anti-SARS-CoV-2 Antibody, Human IgG1 titer by Indirect-ELISA Assay

Immobilized SARS-CoV-2 S1 protein at 2 µg/mL (100 µL/well) can bind Monoclonal Anti-SARS-CoV-2 Antibody, Human IgG1 in 1:50 human serum. Detection was performed using HRP-Conjugated Anti-human IgG antibody with sensitivity of 48 ng/mL.



Anti-SARS-CoV-2 Antibody (ng/mL)	Mean Abs.(OD450)
250	2.719
125	2.523
62.5	1.666
31.25	0.969
15.625	0.562
7.8125	0.331
3.90625	0.227
1.953125	0.164