Solutions For Immunoassays and In Vitro Immunodiagnostics

COVID-19 DOUBLE RBD-ANTIGEN SANDWICH ELISA TOTAL ANTIBODY TEST KIT

For the qualitative determination of human anti-SARS-CoV-2 Spike (S-RBD) total antibodies (IgM/IgG/IgA) in human serum.

Cat#: dAb-eKit-010 **Version 1.0**

This package insert must be read entirely before using this product. **FOR RESEARCH USE ONLY.**

SUMMARY

Coronaviruses (CoV) are a family of single-stranded positive-sense RNA viruses that infect animals and humans. There are seven known types of CoVs. Coronavirus has caused two large-scale pandemics in the last two decades, SARS and MERS outbreaks in 2002 and 2012 respectively occurred. More recently, Coronavirus disease 2019 (COVID-19) is defined as illness caused by a novel coronavirus now called Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). On March 11, 2020, WHO declared COVID-19 a global pandemic, its most recent pandemic declaration since declaring H1N1 influenza a pandemic in 2009. Coronaviruses are composed of several proteins including the Spike (S), Envelope (E), Membrane (M), and Nucleocapsid (N). The Spike protein contains critical Receptor Binding Domain (RBD) for binding to viral receptor. Total antibodies (IgM, IgG and IgA) are the immunoglobulin to be produced in response to the specific antigen and can be primarily detectable during the onset of the disease.

KIT COMPONENTS

Item	Quantity	Specification	Preparation
Coated ELISA Microplate *	1 x 96 well	8 x12 strips	Ready to use
Positive Control *	1 vial	1mL	Ready to use
Negative Control*	1 vial	1mL	Ready to use
Sample Dilution Buffer *	1 bottle	6mL	Ready to use
HRP conjugated SARS-CoV-2 RBD antigen *	1 bottle	11mL	Ready to use
Wash Buffer * 20 ×	1 bottle	50ml	See Preparation
Substrate A *	1 bottle	6mL	Ready to use
Substrate B *	1 bottle	6mL	Ready to use
Stop solution *	1 bottle	6mL	Ready to use
Plate Sealer	4 pieces	NA	Ready to use
Instruction manual	1 copy	NA	Ready to use

* All is stored at 2-8°C

PRINCIPLE OF THE ASSAY

This kit is based on the double-antigen sandwich based enzyme-linked immunosorbent assay (ELISA) technology. It detects total rather than class-specific antibodies to SARS-CoV-2 S-RBD. In double antigen sandwich ELISA kit, the SARS-CoV-2 S-RBD antigen is pre-coated on the plate, which acts as the capturer, and can specifically recognize anti-SARS-CoV-2 antibodies in human serum or plasma specimens. A second S-RBD antigen conjugated to tracer enzyme-horseradish peroxidase (HRP), is used as the detector. The pre-coated and conjugated antigens are bound to the antibodies to SARS-CoV-2 while the unbound components are washed away. Then the specific S-RBD-antibody-antigen-HRP immuno complex are developed. After removal of nonspecific bindings, a HRP substrate solution containing TMB and substrate buffer are added, resulting in the formation of a blue color. Color reaction is stopped by Stop Solution, transforming the blue color to yellow signals, which is quantified by an absorbance microplate reader at 450nm. The color intensity is proportional to the amount of total antibodies to SARS-CoV-2 S-RBD captured inside the wells.

PRECAUTIONS FOR USE

1. After opening and before using, keep plate dry.
2. Before using the Kit, balance the reagents at room temperature at least 30 minutes.
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.

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.

STORAGE AND PREPARATION OF TEST SAMPLES

- Test samples are suggested to be assayed immediately after separation of serum or plasma, or preferably stored frozen (-20°C) in aliquots. Multiple freeze-thaw cycles should be avoided. Duplicate test is recommended.
- Serum or plasma specimens with EDTA, sodium citrate or heparin can be tested. Highly hemolytic specimens are not recommended. Specimens with visible microbial contamination should not be used.
- When required, vortex test serum or plasma samples at room temperature to ensure homogeneity. Then centrifuge samples at 10,000 to 15,000 rpm for 5 minutes prior to assay to remove particulates. Please do not omit this centrifugation step if samples are cloudy and containing particles.

PREPARATION

- **Wash Buffer:** Dilute 50 ml Concentrated Wash Buffer into 950 ml deionized or

distilled water. Put unused solution back at 2-8°C.

ASSAY PROCEDURE

When preparing samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C.

1 Bring all reagents to room temperature before use.

2 Place a sufficient number of pre-coated microwell strips in a holder to run controls and samples in duplicate. *Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

3 Test Configuration as shown in Table

Row	Strip 1	Strip 2	Strip 3
A	Blank Control	SAMPLE 2	SAMPLE 6
B	Blank Control	SAMPLE 2	SAMPLE 6
C	Negative Control *	SAMPLE 3	SAMPLE 7
D	Negative Control *	SAMPLE 3	SAMPLE 7
E	Positive Control *	SAMPLE 4	SAMPLE 8
F	Positive Control *	SAMPLE 4	SAMPLE 8
G	SAMPLE 1	SAMPLE 5	SAMPLE 9
H	SAMPLE 1	SAMPLE 5	SAMPLE 9

4 Label the sample wells as need, always set up 2 Negative Controls, 2 Positive Controls and 2 Blank Controls.

5 Add 50µl sample dilution buffer to each sample well and also add 50µl control wells except blank wells.

6 Add 50µl sample to each sample well. Add 50µl Negative Controls and Positive Controls to set Control wells and gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37°C for 60 minutes.

7 Remove the cover, and wash plate 5 times with Wash buffer and each time let the wash buffer stay in the wells about a minute.

8 Add 100µl HRP-conjugated SARS-CoV-2 recombinant RBD antigen into each well except blank control wells.

9 Seal the plate with a cover and incubate at 37°C for 30 minutes.

10 Wash plate like Step 7 above.

11 Add 50µl of Substrate A and B into each well, respectively. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in dark within 15 minutes. And the shades of obvious blue can be seen in the Positive Controls. Blank wells show no obvious color.

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

13 Read the O.D. absorbance at 450nm in a microplate reader immediately after adding the stop solution 10 minutes (Use the blank well to set zero).

SAMPLE TEST DATA

Serum samples came from COVID-19 infected patients and healthy people were used for evaluated our ELISA kit. All samples were not diluted and added into pre-coated plate as described above. See tested data below.

Sample Test Data of Human Serum by Double Antigen Sandwich ELISA

Control	COVID-19 Patients				Healthy Volunteers			
	Sample	OD	Sample	OD	Sample	OD	Sample	OD
0.059	1#	2.103	9#	0.788	1#	0.05	9#	0.064
0.06	2#	2.491	10#	0.74	2#	0.05	10#	0.057
0.059	3#	2.064	11#	2.18	3#	0.06	11#	0.055
0.06	4#	2.242	12#	2.355	4#	0.05	12#	0.051
2.251	5#	2.554	13#	0.98	5#	0.05	13#	0.05
2.261	6#	1.991	14#	2.68	6#	0.05	14#	0.072
S89	7#	1.152	15#	0.785	7#	0.07	15#	0.05
S90	8#	1.923	16#	0.57	8#	0.05	16#	0.055
Legend Key	BC:Blank Control		NC:Negative Control		PC:Positive Control		S:SAMPLE S1-S90	

PCx=1.791, NCx=0.046.

DATA ANALYSIS

- Calculate the average value of the absorbance of the negative control (NCx) and the Positive Control (PCx).
- Calculate the cutoffs using the followings:
  - Positive cutoff ≥NCx + 0.12
  - Negative cutoff ≤0.10

INTERPRETION OF RESULTS

Determine the interpretation of the sample by comparing the OD to the following table:

Interpretation	Interval	Preparation
Negative	Measured value ≤ Negative cutoff	The sample does not contain the SARS-CoV-2 RBD-related antibody
Positive	Measured value ≥ positive cutoff	The sample does contain the SARS-CoV-2 RBD-related antibody

ANALYTICAL EVALUATIONS

Paramaters	Assay Results
Specificity	550/555 (99.09%)
Sensitivity	118/119 (99.16%)
Cross Reactivity	No any reacted with tested a variety of molecules
Repeatability	The assays were tested in 10 replicates with a CV of OD values, ≤ 8%
Precision	The reads of the same positive sample over 50 different runs performed by at least three batches of Kits along with a negative sample.
Reproducibility	Three lots were tested with the same samples 10 times, ≤ 10%
Stability	At least 12 month at 4°C

SUMMARY OF ASSAY PROCEDURE

1. Add samples and controls to each assigned well and Incubate at 37°C for 60 mins.
2. Aspirate and wash each well three times.
3. Add Detectioantigen solution to each well and Incubate at 37°C for 30 mins.
4. Aspirate and wash each well five times.
5. Add Substrate solution to each well and Incubate at 37°C for 15 mins.
6. Add Stop solution to each well and Measure absorbance of each well at 450 nm.
7. Calculation and Interpretation results