

## Total antibody (IgG and IgM) to SARS-COV-2 S-RBD protein ELISA kit datasheet

For the qualitative detection of total antibody (IgG and IgM) to SARS-COV-2 S-RBD protein in serum and plasma.

**For research use only, not for clinical diagnosis.**

### general information

Catalogue Number	KE30006
Product Name	Total antibody (IgG and IgM) to SARS-COV-2 S-RBD protein Elisa kit (Antibody coated)
Species cross-reactivity	Total antibody (IgG and IgM) to SARS-COV-2 S-RBD protein
Range (calibration Range)	6.25-200 ng/mL
Tested applications	Qualitative detection ELISA

### kit components & storage

Microplate - anti-human antibody coated 96 - well Microplate (8 well × 12 strips)	1 plate	<b>Unopened Kit:</b> Store at 2-8°C for 6 months or -20°C for 12 months
Standard - 200 ng/bottle; lyophilized*	1 bottle	
HRP-conjugated S-RBD protein (100X) - 120 µL/vial	1 vial	<b>Opened Kit:</b> All reagents could be stored at 2-8°C for 7 days <b>Please use a new standard for each assay</b>
Sample Diluent PT 4B1 - 30 mL/bottle	2 bottles	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	2 pieces	

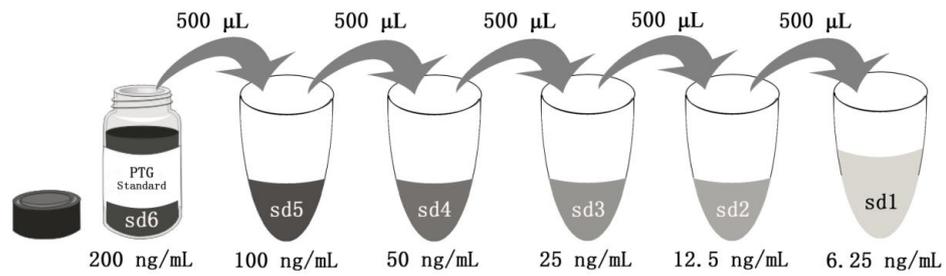
**NB: Do not use the kit after the expiration date.**

**This kit is for research use only.**

Sample Diluent **PT 4B1** is for standard and samples.

Detection Diluent is for HRP-conjugated S-RBD protein.

\*Add 1 mL Sample Diluent PT 4B1 in standard. This reconstitution gives a stock solution of 200 ng/mL.



Add # $\mu\text{L}$ of Standard diluted in the previous step	—	500 $\mu\text{L}$				
# $\mu\text{L}$ of Sample Diluent PT 4B1	1000 $\mu\text{L}$	500 $\mu\text{L}$				
	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## product description

KE30006 is a quantitative measurement of total antibody (IgG and IgM) to SARS-COV-2 S-RBD protein in serum or plasma. Anti-human antibody has been pre-coated onto microplate well. The samples or standard are added to the well, after incubation the wells are washed and a horseradish peroxidase conjugated S-RBD protein is added to each well. Producing an complex "anti-human antibody and sample and SARS-CoV-2 S-RBD protein-HRP conjugated". after incubation the wells are washed, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

## background

A promising target for both diagnosis and therapeutics treatments of the new disease named COVID-19 is the coronavirus (CoV) spike (S) glycoprotein. The spike protein, which is responsible for the "corona" (Latin word for crown) appearance in all coronaviruses, is a type I glycoprotein that has an especial role in the interaction between the virus and the host cell. This protein attaches itself to specific cellular receptors and suffers a conformational change that enables the fusion of the virus and the cell (1). Studies have shown that the SARS-CoV-2 S-RBD protein interacts strongly with the Angiotensin-converting enzyme 2 (ACE2). S-RBD protein in order to enlighten the binding epitopes of these Abs. Because of the conservation of S-RBD protein sequence and its strong immunogenicity, the S-RBD protein of coronavirus is chosen as a diagnostic tool. COVID-19 antibodies can be produced by a host immune system following exposure to SARS-CoV-2. IgG antibodies are also known as immunoglobulins IgG, respectively, and are among the antibody isotypes produced by vertebrate immune systems.

## reagent preparation

### A. HRP-conjugated S-RBD protein

Dilute **100X HRP-conjugated S-RBD protein** 1:100 using **Detection Diluent** prior to assay. Suggested 1:100 dilution: 10  $\mu$ L **HRP-conjugated anti-N protein** + 990  $\mu$ L **Detection Diluent**.

### B. Wash Buffer

Allow the **20X Wash Buffer** to reach room temperature before use. Dilute entire 30 mL of **20X Wash Buffer concentrate** with 570 mL deionized, distilled water. If crystals remain in the concentrate, warm to 37°C and mix gently until the crystals have dissolved completely. Store at 2–8°C.

## sample preparation

The plasma sample may require proper dilution to fall within the range of the assay. A range of dilutions like 1:100 is suggested according to the individual samples. Severe hemolytic samples should not be used.

## safety notes

This product is sold for lab research and development use ONLY and not for use in humans or animals.

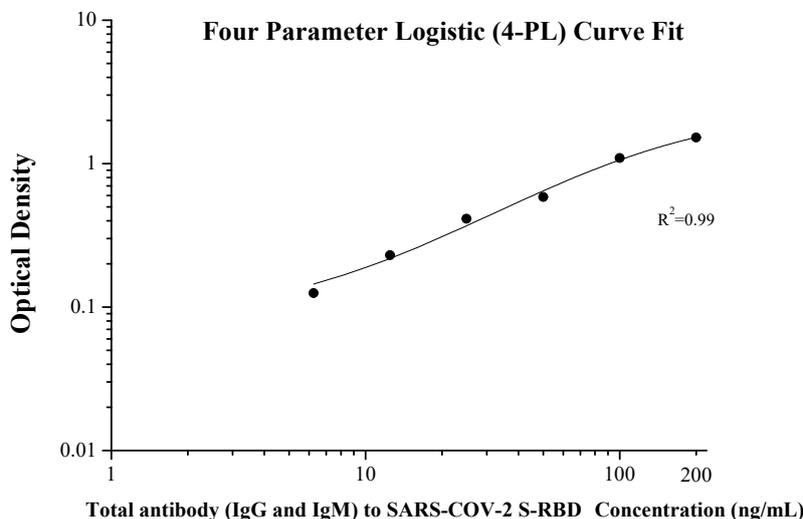
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 $\mu$ L	<b>30 min</b>	4 times	Cover Wells incubate at RT (25 °C)
2	Diluent 1x <b>HRP-conjugated S-RBD protein</b> Solution	100 $\mu$ L	30 min	4 times	Cover Wells incubate at RT (25 °C)
3	TMB Substrate	100 $\mu$ L	10-15 min	Do not wash	Cover Wells incubate at RT (25 °C)
4	Stop Solution	100 $\mu$ L	0 min	Do not wash	-
5	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

## typical data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D	Correcte
0	0.077	—
6.25	0.202	0.125
12.5	0.307	0.23
25	0.489	0.412
50	0.663	0.586
100	1.169	1.092
200	1.596	1.519

## assay procedure in summary

Please Note:

- Equilibrate all reagents and samples at room temperature before use.
  - Gently mix each reagent before use.
  - It is recommended to assay all standards, controls, and samples in duplicate
1. Place a sufficient number of microwell strips in a holder to run controls and samples in duplicate.
  2. Add 100  $\mu$ L each of standard and 1: 100 diluted samples into the microwells.
  3. Mix gently and cover the plate with one plate cover seal. Incubate at room temperature (25 $^{\circ}$ C) for 30 minutes.
  4. Remove the plate cover seal. Aspirate the contents of each well. Wash each well 4 times by dispensing 350 $\mu$ L of diluted 1Xwash solution into each well.
  5. Add 100  $\mu$ L of the 1x HRP-conjugated S-RBD protein into the microwells.
  6. Mix gently and cover the plate with one plate cover seal. Incubate at room temperature (25 $^{\circ}$ C) for 30 minutes with a plate cover seal. Aspirate the contents of each well. Wash each well 4 times by dispensing 350 $\mu$ L of diluted wash solution into each well.
  7. Add 100 $\mu$ L of the substrate into the microwells.
  8. Incubate at room temperature (25 $^{\circ}$ C) for 10-15 minutes and add 100 $\mu$ L of stop solution into each of the microwells.
  9. Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.

## **data analysis**

Average the duplicate readings for each standard and sample and subtract the average zero standard absorbance (obtained from the average of the “sd0” readings). The best-fit standard curve can be determined by regression analysis using four-parameter logistic curve fit (4-PL). As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best-fit curve through the points on the graph. The data may be linearized by plotting the log of the Standard concentrations versus the log of the OD readouts. The best-fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

## **references**

1. YZumla, A., Chan, J. F. W. et al. (2016). Coronaviruses-drug discovery and therapeutic options. *Nat. Rev. Drug Discov.* 15, 327–347.
2. Penghui Yang, Xiliang Wang. (2020) COVID-19: A New Challenge for Human Beings, *Cell Mol Immunol.* 17(5):555-557.