

IgM for SARS-CoV-2 S-RBD ELISA kit datasheet

For the qualitative detection of IgM for SARS-CoV-2 S-RBD in serum or plasma.

For research use only, not for clinical diagnosis.

general information

Catalogue Number	KE30004
Product Name	IgM for SARS-CoV-2 S-RBD ELISA Kit (Antigen coated)
Species cross-reactivity	Human IgM for SARS-CoV-2 S-RBD
Tested applications	Qualitative detection ELISA

kit components & storage

Microplate - S-RBD protein coated 96 - well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Protein standard - 100 ng/bottle; lyophilized*	1 bottle	Store at 2-8°C for six months
HRP-conjugated anti-human IgM antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 4B1 - 30 mL/bottle	2 bottles	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	2 pieces	

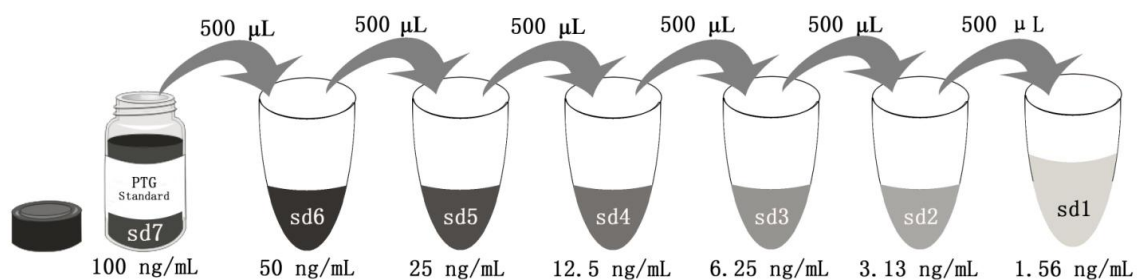
NB: Do not use the kit after the expiration date.

This kit is for research use only.

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

Detection Diluent is for HRP-conjugated anti-human IgM antibody.

*Add 1 mL Sample Diluent PT 4B1 in protein standard. This reconstitution gives a stock solution of 100 ng/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 4B1	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE30004 is a qualitative measurement of the human IgM for SARS-CoV-2 S-RBD in serum and plasma. The principle of the kit is indirect ELISA. S-RBD Recombinant has been pre-coated onto microplate well. The samples are added to the well, after incubation the wells are washed and a horseradish peroxidase conjugated anti-Human IgM is added to each well. Producing a complex "Recombinant Protein–human anti-S-RBD IgM antibody-HRP conjugated second antibody". 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 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. IgM antibodies are also known as immunoglobulins IgM, respectively, and are among the antibody isotypes produced by vertebrate immune systems. The ELISA microplate is coated with the SARS-CoV-2 S-RBD protein. The coated S-RBD protein binds with COVID-19 IgM S-RBD antibodies in the serum and plasma sample.

reagent preparation

A. HRP-conjugated secondary antibody

B. Dilute **HRP-conjugated anti-human IgM antibody** 1:100 using **Detection Diluent** prior to assay. Suggested 1:100 dilution: 10 μ L **100X HRP-conjugated anti-human IgM antibody** + 990 μ L **Detection Diluent**.

C. 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.

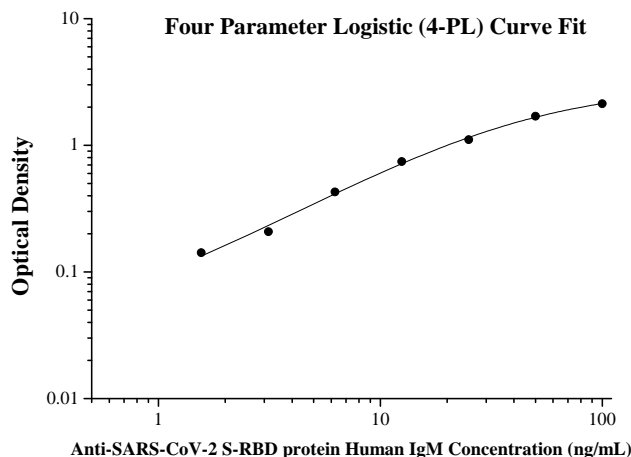
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 μ L	30 min	4 times	Cover Wells incubate at room temperature (25°C)
2	Diluent 1X HRP-conjugated anti-human IgM antibody Solution	100 μ L	30 min	4 times	Cover Wells incubate at room temperature (25°C)
3	TMB Substrate	100 μ L	10-15 min	Do not wash	Cover Wells incubate at room temperature (25°C)
4	Stop Solution	100 μ 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	Average	Corrected
0	0.014	0.014	—
	0.013		
1.56	0.15	0.156	0.142
	0.161		
3.13	0.288	0.285	0.208
	0.282		
6.25	0.483	0.505	0.428
	0.526		
12.5	0.783	0.819	0.742
	0.854		
25	1.166	1.184	1.107
	1.202		
50	1.797	1.774	1.697
	1.75		
100	2.193	2.207	2.193
	2.22		

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 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 μ 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 μ L of diluted 1Xwash solution into each well.
 5. Add 100 μ L of the 1x **HRP-conjugated anti-human IgM antibody** Solution 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 μ L of diluted wash solution into each well.
 7. Add 100 μ L of the substrate into the microwells.
 8. Incubate at room temperature (25 $^{\circ}$ C) for 10-15 minutes and add 100 μ 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.

references

1. Walls A.C. et al. (2020) Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell. 2020.02.058.
2. Xu X., et al. Hao P. (2020) Evolution of the novel coronavirus from the ongoing wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci. China Life Sci. 2020;63(3):457–460.