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Antibodies | ELISA kits | Proteins

Anti-SARS-CoV-2 N protein Human IgG ELISA kit datasheet

For the qualitative detection of Anti-SARS-CoV-2 N protein Human IgG ELISA Kit in serum and plasma.

For research use only, not for clinical diagnosis.

general information

Catalogue Number	KE30001
Product Name	Anti-SARS-CoV-2 N protein Human IgG ELISA Kit (Antigen coated)
Species cross-reactivity	Anti-SARS-CoV-2 N protein Human IgG
Range (calibration Range)	8 - 128 ng/mL
Tested applications	Qualitative detection ELISA

kit components & storage

Microplate - N protein coated 96 - well Microplate (8 well × 12 strips)	1 plate	**Unopened Kit**: Store at 2-8°C for 6 months or -20°C for 12 months **Opened Kit**: All reagents could be stored at 2-8°C for 7 days **Please use a new standard for each assay**
Standard - 128 ng/bottle; lyophilized*	1 bottle	
HRP-conjugated anti-human IgG antibody (100X) - 120 µL/vial	1 vial	
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	

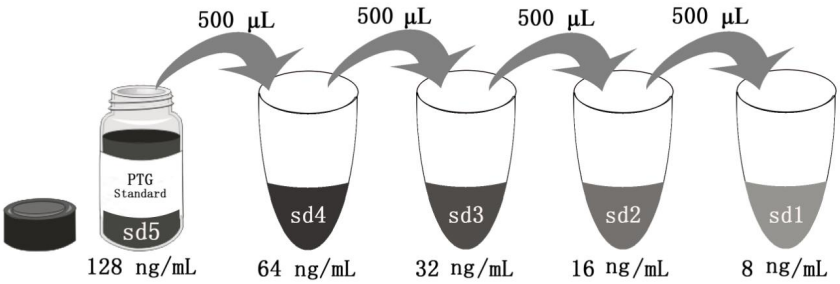
NB: Do not use the kit after the expiration date.

This kit is for research use only.

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

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

*Add 1 **mL** Sample Diluent PT 4B1 in standard. This reconstitution gives a stock solution of 128 ng/mL.



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

product description

KE30001 is a quantitative measurement of Anti-SARS-CoV-2 N protein Human IgG in plasma. The principle of the kit is indirect ELISA. N protein recombinant protein 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 anti-Human IgG is added to each well. Producing an complex “Recombinant Protein–human anti-NP IgG antibody-HRP conjugated 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

Coronaviruses are enveloped viruses with a positive-sense RNA genome and with a nucleocapsid of helical symmetry. Coronavirus nucleoproteins localize to the cytoplasm and the nucleolus, a subnuclear structure, in both virus-infected primary cells and in cells transfected with plasmids that express N protein. Coronavirus N protein is required for coronavirus RNA synthesis and has RNA chaperone activity that may be involved in template switch. Nucleocapsid protein is a most abundant protein of coronavirus. During virion assembly, N protein binds to viral RNA and leads to formation of the helical nucleocapsid. Nucleocapsid protein is a highly immunogenic phosphoprotein also implicated in viral genome replication and in modulating cell signaling pathways. Because of the conservation of N protein sequence and its strong immunogenicity, the N 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 and IgM antibodies are also known as immunoglobulins IgG and IgM, respectively, and are among the antibody isotypes produced by vertebrate immune systems. The ELISA microplate is coated with the SARS-CoV-2 nucleocapsid (N) protein. The coated N protein binds with COVID-19 IgG N antibodies in the serum sample.

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reagent preparation

A. **HRP-conjugated secondary antibody**

Dilute **100X HRP-conjugated anti-human IgG antibody** 1:100 using **Detection Diluent** prior to assay. Suggested 1:100 dilution: 10 µL **HRP-conjugated anti-human IgG antibody** + 990 µL **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 µL	**30 min**	4 times	Cover Wells incubate at room temperature (25 °C)
2	Diluent 1x **HRP-conjugated anti-human IgG antibody** Solution	100 µL	30 min	4 times	Cover Wells incubate at room temperature (25 °C)
3	TMB Substrate	100 µL	10-15 min	Do not wash	Cover Wells incubate at room temperature (25 °C)
4	Stop Solution	100 µL	0 min	Do not wash	-
5	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

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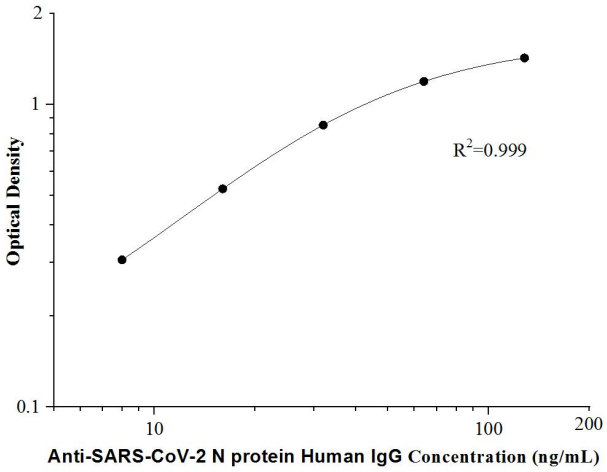
typical data

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	Correcte
0	0.012	0.012	—
	0.012		
8	0.316	0.319	0.307
	0.321		
16	0.521	0.539	0.527
	0.556		
32	0.84	0.867	0.855
	0.894		
64	1.176	1.204	1.192
	1.231		
128	1.406	1.437	1.425
	1.467		

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 µL 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℃) for 30 minutes.
4. Remove the plate cover seal. Aspirate the contents of each well. Wash each well 4 times by dispensing 350µL of diluted 1Xwash solution into each well.
5. Add 100 µL of the 1x HRP-conjugated Anti-human IgG secondary antibody into the microwells.
6. Mix gently and cover the plate with one plate cover seal. Incubate at room temperature (25℃) for 30 minutes with a plate cover seal. Aspirate the contents of each well. Wash each well 4 times by dispensing 350µL of diluted wash solution into each well.
7. Add 100µL of the substrate into the microwells.
8. Incubate at room temperature (25 ℃) for 10-15 minutes and add 100µL 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.

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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.

sensitivity

The minimum detectable dose of Anti-SARS-CoV-2 N protein Human IgG is 0.026 ng/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of standard in human plasma and diluted with **Sample Diluent PT 4B1** to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:50)

		Human plasma
1:2	Average% of Expected	105
	Range (%)	89-115
1:4	Average% of Expected	97
	Range (%)	86-108
1:8	Average% of Expected	102
	Range (%)	85-111
1:16	Average% of Expected	114
	Range (%)	92-128

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.