COVID-19 (SARS CoV-2) IgG ELISA Kit

Catalog # PMK-90001 version 3.0

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ProMab
Biotechnologies, Inc.

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Background

A new coronavirus, SARS-CoV-2 causing coronavirus disease 19 (COVID-19), has recently appeared to cause a human global pandemic. Whereas molecular diagnostic tests such as qRT-PCR detecting viral RNA were rapidly developed, the serologic assays detecting antibodies in patients are urgently needed. Validated serologic assays are important for several reasons: 1. for detection of the viral reservoir and epidemiological studies, for detection of asymptomatic cases and estimation of mortality; 2. for evaluation of vaccine development; 3. for development of therapeutic antibodies. The tests that not only detect the virus but also detect immunity to SARS-CoV-2 are crucial to overcome the pandemic, to limit economic damage, get people back to work, reopen borders - and those whose immunity can be demonstrated should be able to return to work, without risk (1). The Food and Drug administration (FDA) already relaxed assessment criteria for different SARS-CoV2 detection kits.

With antibody detection kits, there is versatility in analyzing the results. You can show that the person overcame COVID-19 disease and has developed antibodies (which means they are immune and can resume working), something that cannot be shown with a PCR kit. It can show that vaccines are working in clinical trials or be used in contact tracing weeks or longer after a suspected infection in an individual.

Antibody tests require knowledge of specific viral proteins which cause immune system to respond and to trigger production of antibodies, detect virus and neutralize the virus. These viral proteins are produced in the lab as recombinant proteins and are used in this ELISA kit to detect antibodies present in the patient serum. ProMab developed several ELISA kits, with this example using the Nucleocapsid protein of SARS-CoV-2 to detect antibodies from human serum.

Assay Principle

The ProMab SARS-CoV-2 IgG ELISA kit was designed, developed, and produced for the qualitative detection of IgG antibodies specific for the SARS-COV-2 nucleocapsid (N) protein. Human serum samples are added to a 96-well microplate that has been pre-coated with SARS-CoV-2 recombinant N protein. During 30 minutes of incubation where N protein-specific IgG antibody in the serum are “trapped” and bound to the plate, the other non-specific antibodies and proteins are removed with a washing step. Next, horseradish peroxidase (HRP)-labeled goat anti-human IgG Fc is added to each well, and binds to the Fc portion of the N protein-specific IgG for 30 minutes. After another washing step to remove the unbound HRP-labeled antibody, an HRP substrate is added to the wells. Over the course of 20 minutes, the green/blue TMB substrate is converted by HRP into a yellow colored product. The reaction is stopped with sulfuric acid and the intensity of the colored product is detected using a spectrophotometric microplate reader.

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Product Details

PRODUCT NAME	ProMab's SARS-CoV-2 Nucleocapsid Protein IgG ELISA Kit		
COMPONENTS AND STORAGE			
Part #	Items and Description	Amount	Storage
PMK-2020401	Microplate coated with SARS-CoV-2 recombinant N protein	1 x 96 well microplate	2-8°C upon receipt
PMK-2020402	SARS-CoV-2 IgG Sample Dilution Buffer Ready-to-use sample dilution buffer (1%BSA in PBS).	1 x 35 mL	2-8°C upon receipt
PMK-2020403	HRP labeled goat anti-human IgG antibody Ready-to-use HRP labeled polyclonal goat anti-human IgG Fc antibody.	1 x 6 mL	2-8°C upon receipt
PMK-2020404	ELISA Wash Concentrate (50X) 50X concentrated buffer with surfactant. Preparation: Add to 735 mL distilled water and mix well before use.	1 x 15 mL	2-25°C upon receipt
PMK-2020405	TMB Substrate Ready-to-use Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.	1 x 10 mL	2-8°C upon receipt, protect from light
PMK-2020406	Stop Solution Ready-to-use 1.0 M sulfuric acid.	1 x 10 mL	2-25°C upon receipt
PMK-2020407	SARS-CoV-2 IgG Negative Control Ready-to-use solution containing non-specific IgG.	1 x 0.5 mL	2-8°C upon receipt
PMK-2020408	SARS-CoV-2 IgG Positive Control Ready-to-use solution containing N protein-specific IgG.	1 x 0.25 mL	2-8°C upon receipt
REQUIRED MATERIALS AND EQUIPMENT NOT PROVIDED	• Human serum samples • Microplate reader capable of measuring absorbance at 450nm • Microplate washer (manual or automated) • 96-well microplate for diluting samples • Distilled water • Precision pipettors (10 µL, 100 µL, 200 µL and 1000 µL and pipette tips) • Multichannel pipettors • Disposable reagent reservoir • Paper towels • Timer • Refrigerator/freezer to store samples and kit components		
RESTRICTIONS	For Research Use Only		

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Application Details

SERUM COLLECTION AND STORAGE

Serum samples should be collected using conventional methods as outlined by the CDC and NCCLS for preventing transmission of blood-borne pathogens.

Specimens storage after collection:

- Store up to 72 hours at 4°C.
- Store at -20°C or below if testing cannot be performed promptly.

PROCEDURAL GUIDELINES

Instrument compatibility

A variety of microplate reader instruments can be used with this kit, with the minimum requirement being a spectrophotometric microplate reader capable of reading absorbance at 450 nm.

ELISA Procedure:

1. Preparation of reagents
   A. Equilibrate the pre-coated test strip(s) (PMK-2020401) and all other reagent to room temperature (18°C-25°C) for at least 30 minutes.
   B. Calculate the total number of wells needed, including wells for the positive control (PMK-2020408) and negative control (PMK-2020407) according to the experimental design tables (below). Store the unused strips at 2-8°C.
   C. Prepare 1X Washing Buffer Concentrate by adding 15 mL of the Wash Concentration (PMK-2020404) with 735 mL of distilled water. Mix well and leave at room temperature.
2. Preparation of samples. It is recommended to test all samples in duplicate. Dilute testing samples with diluent buffer (PMK-2020402) at 1:200. e.g.: Mix 10 µL of sample with 90 µL buffer, then dilute 10 µL of this diluted sample into 190 µL buffer and mix well.

Example Experimental Design Table (12 strips)

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	S2	S6	S10	S14	S18	S22	S26	S30	S34	S38	S42
B	NC	S2	S6	S10	S14	S18	S22	S26	S30	S34	S38	S42
C	PC	S3	S7	S11	S15	S19	S23	S27	S31	S35	S39	S43
D	PC	S3	S7	S11	S15	S19	S23	S27	S31	S35	S39	S43
E	BLK	S4	S8	S12	S16	S20	S24	S28	S32	S36	S40	S44
F	BLK	S4	S8	S12	S16	S20	S24	S28	S32	S36	S40	S44
G	S1	S5	S9	S13	S17	S21	S25	S29	S33	S37	S41	S45
H	S1	S5	S9	S13	S17	S21	S25	S29	S33	S37	S41	S45

Example Experimental Design Table (3 strips)

	1	2	3
A	NC	S2	S6
B	NC	S2	S6
C	PC	S3	S7
D	PC	S3	S7
E	BLK	S4	S8
F	BLK	S4	S8
G	S1	S5	S9
H	S1	S5	S9

Legend
NC= Negative Control
PC= Positive Control
S= Sample#

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Application Details

PROCEDURAL GUIDELINES

3. Add samples. Add the pre-diluted samples along with the positive and negative control to the designated wells (50uL per well). Cover the plate with the provided adhesive film cover and in cubate the plate at 37°C for 30 minutes.
4. Wash plates. Remove the adhesive sealer film and quickly decant the samples into a waste container. Wash each well with 200uL of 1X Washing Buffer (prepared in Step 1C) three times. Alternatively, instead of manual washing, an automated microplate washer can be used.
5. Add secondary antibody. Add 50uL of HRP-labeled goat anti-human IgG antibody (PMK-2020403) to each well. Cover the plate and incubate at 37°C for 30 minutes.
6. Wash each well as done in Step 4, repeating for five times instead of three.
7. Add substrate. Add 50uL of the TMB substrate (PMK-2020405) to each well and tap the plate gently to mix. Incubate the plate in the dark at room temperature for 20 minutes. Note: the HRP substrate must be warmed to room temperature in advance.
    a. Reaction termination. Check color formation occasionally to avoid overdevelopment (the negative control wells should be colorless or very light blue). After ~20 minutes, add 50uL of Stop Solution (PMK-2020406) into each well. Mix well by gently tapping the plate.
8. Read absorbance. Read the absorbance with a microplate reader at 450nm within 10 minutes after adding the stop solution.

INTERPRETATION OF RESULTS

1. Each experimental result is used independently, and the result is judged by the cut-off value.
2. Calculate the cut-off value:
    a. If the average absorbance (OD value) of the negative control wells is ≤ 0.2, the cut-off value is 0.2.
    b. If the average absorbance (OD value) of the negative control wells is > 0.2, the cut-off value is the average OD value.
3. Judgement of results:
    a. Negative result: The sample's absorbance (OD value) is ≤ the cut-off value.
    b. Positive result: The sample's absorbance (OD value) is ≥ the cut-off value X 2.
4. Each laboratory should establish its own cut-off value. The below cut-off values are for reference only.

Interpretation	Interval	Results
Negative	Sample OD value is ≤ cut-off value	The sample does not contain SARS-CoV-2 N protein-specific IgG related antibodies
Positive	Sample OD value is ≥ the cut-off value X 2	The sample contains SARS-CoV-2 N protein-specific IgG related antibodies
Other	Sample OD value is > cut-off but ≤ cut-off value X 2	Re-test the sample. If still inconclusive, consider other clinical tests.

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PERFORMANCE CHARACTERISTICS

Limit of Detection

No international standardized units are available for SARS-CoV-2. A positive sample was serially diluted, and the limit of detection was determined to be ≤ 5 U/mL.

Repeatability

The SARS-CoV-2 IgG Positive Control was tested in 10 replicates with a CV less than 15%.

Reproducibility

Three lots were tested with the same samples 10 times with a CV less than 20%.

Class Specificity

The ProMab SARS-CoV-2 IgG ELISA Kit does not show any cross-reaction to N protein-specific IgM.

Assay Validation

The ProMab SARS-CoV-2 IgG ELISA kit was used to test 22 clinical samples that were previously tested by qRT-PCR. The samples were run in duplicate and the OD value shown is the average between the two readings.

Specimen #	qRT-PCR Status	OD Value	Specimen #	qRT-PCR Status	OD Value
1	Negative	0.235	12	Positive	2.302
2	Positive	0.2795*	13	Negative	0.3125
3	Positive	0.069*	14	Negative	0.1055
4	Negative	0.1335	15	Negative	0.0965
5	Negative	0.087	16	Negative	0.095
6	Negative	0.1395	17	Positive	0.448
7	Negative	0.1085	18	Positive	1.337
8	Negative	0.0965	19	Positive	0.8365
9	Positive	2.178	20	Positive	1.4885
10	Positive	2.254	21	Positive	1.4125
11	Positive	2.3635	22	Positive	1.436

*Samples were collected day of positive confirmation. See Assay Limitations #2.

ASSAY LIMITATIONS

1. The ProMab SARS-CoV-2 ELISA kit is intended only for the qualitative detection of N protein-specific antibodies. Test results should not be the sole basis for clinical diagnosis and treatment. The patient's clinical symptoms must be considered in conjunction with other tests.
2. In the first week of infection with SARS-CoV-2, patients' samples may be negative in this assay. In addition, negative results may occur when patients have a compromised immune system, have a disease that affects immunity, have failure of important systemic organs, or use drugs that suppress immune function.
3. Previous infection with SARS-CoV-1 or other coronavirus strains may cause a positive re sult, due to cross-reactivity between the strains' N proteins.
4. Bacterial or fungal contamination of samples or reagents, or cross-contamination between reagents, may cause erroneous results.
5. Deionization of water with polyester resins may inactive the HRP enzyme.
6. Personnel should be trained and familiar with ELISA procedures and prior to performing the assay.

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QUALITY CONTROL	To assure the validity of the results, each assay must include both negative and positive controls. The average value of the absorbance of the negative control should be less than 0.25, and the average value of the absorbance of the positive control should not be less than 0.40. ProMab also recommends that all assays include the laboratory's own controls in addition to those provided with this kit.
SAFETY AND TECHNICAL PRECAUTIONS	The ProMab SARS-CoV-2 ELISA kit is designed for research use only, for the purpose of in vitro detection of SARS-CoV-2 N protein-specific antibodies. The ProMab SARS-CoV-2 ELISA kit must not be used for other purposes. Source material contains bovine serum albumin (BSA) derived in the contiguous 48 United States. The BSA was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Do not mix reagents between different lots. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. Avoid contact with eyes, on skin, or clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Practice Good Laboratory Practices.
REFERENCES	1. CDC (2020). Transmission of Novel Coronavirus (COVID-19) 2. Chenjia Yuan , Shi Jinsong , Qiudong An , Liu Chang , Li Xin , Qiang , Ruanji Shou , mountains . Wuhan 2019 Bioinformatics coronavirus genome analysis [J / OL]. Bioinformatics: 1-10 [2020-02-10]. 3. Xu, X., Chen, P., Wang, J., Feng, J., Zhou, H., Li, X., Hao, P. (2020). Evolution of the novel coronavirus from the ongoing Wuhan outbreak and mode protein for risk of human transmis sion. Science China Life Sciences 10.1007/s11427-020-1637-5 4. Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Shi, Z. (2020). A pneumo nia outbreak associated with a new coronavirus of probable bat origin. Nature. doi: 10.1038/ s41586-020-201