QUICK PROFILE™ 2019-nCoV IgM Enzyme Immunoassay Test Kit

Catalog Number: 78109

Enzyme Immunoassay for the detection of IgM antibodies to SARS-CoV-2 virus in human serum or plasma

INTENDED USE

The animal reservoir of the virus has not yet been identified, but the genome of CoV-19 is so similar to bat coronavirus (98%), reinforcing the presumption that the virus was transmitted by an animal in the shopping center in Wuhan. With regard to genomic similarity, the virus differs from its predecessors, namely SARS (79%) and MERS (50%). As indicated by genetic data, COVID-19 pathogen is classified as a member of the beta-coronavirus genus, and can bind to the angiotensin-converting enzyme 2 receptor in humans.
Human to human transmission via either respiratory droplets or close contact was initially proposed as the main routes of transmission of the pathogen based on experience gained in the previous two epidemics caused by coronaviruses (MERS-CoV and SARS-CoV)(8). According to the World Health Organization (WHO) report, 2019-nCoV is a unique virus that causes respiratory disease, which spreads via oral and nasal droplets.
The cloning and sequencing of CoV19 genome have lead to the development of serological tests for the detection of IgM anti CoV- 19.

PRINCIPLE

Microplates are coated with CoV19-specific immuno-dominant recombinant antigens. The solid phase is first treated with the diluted sample and CoV19 IgM are captured, if present, by the antigens. After washing out all the other components of the sample, in the 2nd incubation bound CoV19 IgM are detected by the addition of anti hIgM antibody, labeled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the Chromogen/Substrate solution, generates an optical signal that is proportional to the amount of anti CoV19 IgM antibodies present in the sample.
Interference due to IgG and RF in samples is blocked directly into the well by a Neutralizing Reagent.

PRECAUTION

Safety Precautions

1. All the reagents contained in the kit are for in vitro diagnostic use only.
2. Do not use the kit or reagents after the expiration date stated on labels.
3. Do not mix reagents of different lots.
4. Procedures should be performed carefully in order to obtain reliable results and clinical interpretations.
5. Bring all the reagents to room temperature for at least 60 minutes, before the test is started.
6. Avoid any contamination of reagents when taking them out of vials. We recommend to use automatic pipettes and disposable tips. When dispensing reagents, do not touch the wall of microplate wells with tips, in order to avoid any cross-contamination.
7. In the washing procedure, use only the Washing Solution provided with the kit and follow carefully the indications reported in the "*Washing Instructions*" section of this insert.
8. Ensure that the Chromogen/Substrate does not come in contact with oxidizing agents or metallic surfaces; avoid any intense light exposure during the incubation step or the reagent preparation.
9. Put the reagents in a glass or plastic disposable container, washed with sulfuric acid 1N, then with deionized water, before use.
10. Samples and materials potentially infective have to be handled with care as they could transmit infection.
All objects coming in direct contact with samples and all residuals of the assay should be treated as potentially infective and properly disposed of. Best procedures for inactivation are treatments with autoclave at 121 °C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 30 minutes. This last method can be used for the treatment of the liquid waste after that it has been neutralized with NaOH.
11. Avoid any contact of liquids with skin and mucous membrane. Use always protective gloves, glasses and laboratory coats, according to the safety regulations.
12. Some reagents of the kit contain sodium azide which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water.

Technical Precautions

1. At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag.
2. Distribution and incubation times should be the same for all the wells; avoid long interruptions among the different steps of the assay.
3. It is suggested to eliminate the excess of washing solution from wells by blotting them gently on a paper adsorbent pad.
4. The color developed in the last incubation is stable for a maximum of 1 hour in the dark.
5. We recommend reading the microplate at 450 nm (reading filter) and subtracting the blank at 620 - 630 nm (blanking filter). Blank the reader on A1 well.

SHELF LIFE OF THE KIT

The shelf life of the kit is 15 months from the production date. The validity of the shelf life is intended for a product stored according to the instructions. The expiration date is indicated on the external label of the package.
Note – Don't use the product after the expiration date.

STORAGE AND STABILITY OF THE REAGENTS

1. The kit has to be stored at 2 – 8 °C and used before the expiration date declared on the external label.
2. The pouch containing the microplate has to be brought to room temperature before opening. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag. Close hermetically the pouch and store again at 2 - 8 °C. If stored properly, strips are stable for 2 months from opening.
3. The diluted Washing solution, at room temperature, is stable for 1 week.
4. The Chromogen and Substrate are stable until the expiration date of the kit.
5. The other reagents can be used every time, if stored at 2 – 8 °C and handled carefully for avoiding contamination.

MATERIALS PROVIDED

Strip Microplate (REF COMM01) **-** Microplate(s) of 8 x 12 strips of breakable wells activated with recombinant CoV19 antigens. The microplates are sealed in an aluminium pouch in presence of desiccant bag.

no. of microplates	REF 1089	no. 1	REF 1089.1	no. 2

Positive Control - *Ready to use.* Buffered solution of chimeric base reactive for CoV IgM. It contains 0.02% gentamicin sulfate and 0.09% Kathon as preservatives and Coomassie brilliant blue as colouring agent.

Volume	REF COMCP01	0.6 ml	REF COMCP01.1	1.2 ml

Negative Control - *Ready to use.* Buffered solution of serum base not reactive for anti-CoV19 antibodies. It contains 0.09% sodium azide and 0.09% Kathon as preservatives and Coomassie brilliant blue as colouring agent.

Volume	REF COMCN01	1.0 ml	REF COMCN01.1	2.0 ml

Sample Diluent - Proteic solution for the dilution of samples that contains stabilizers, 0.09 % thimerosal and 0.09 % Kathon as preservatives and Coomassie brilliant blue as colouring agent.

Volume	REF COMDC01	50.0 ml	REF COMDC01	2 X 50.0 ml

Neutralizing Reagent - Proteic solution for the neutralization of IgG and RF in samples.

It contains a detergent, proteic stabilizers, 0.09 % sodium azide and 0.09 % Kathon as preservatives and Coomassie brilliant blue as colouring agent.
Volume REF COMRN01 5.0 ml REF COMRN01.1 10.0 ml

Washing Solution – *To dilute before use*. Solution 25x concentrated that contains Imidazole buffer and surface-active agent.
Volume REF SL01 50.0 ml REF SL01 2 x 50.0 ml

Conjugate – *To dilute before use.* Solution of proteic buffer, 20x concentrated, that contains anti-human IgM antibodies, labelled with HRP, proteic stabilizers, 0.02% gentamicin sulfate and 0.09% Kathon as preservatives.
Volume REF COMTE01 0.7 ml REF COMTE01.1 1.4 ml

Conjugate Diluent – Buffered proteic solution, for the dilution of the concentrated Conjugate that contains proteic stabilizers, 0.02% gentamicin sulfate and 0.09 % Kathon as preservatives and Ponceau red as colouring agent.
Volume REF COMDT01 14.0 ml REF COMDT01.1 28.0 ml

Chromogen – *To mix with Substrate.* Solution of 3,3',5,5' tetramethylbenzidine (TMB), activators and stabilizers, in a phosphate/citrate buffer.
Note: Store protected from light.
Volume REF TA01 7.0 ml REF TA01.1 14.0 ml

Substrate – *To mix with Chromogen.* Solution that contains hydrogen peroxide (H_2O_2), activators and stabilizers, in a phosphate/citrate buffer.
Volume REF TB01 7.0 ml REF TB01.1 14.0 ml

Stop Solution – Solution of 0.3 M sulphuric acid.
Note: handle with care.
Volume REF SA01 10.0 ml REF SA01.1 20.0 ml

Plate Sealer (REF 300400) **-** Transparent plate sealer to cover microplates during the incubation at 37 °C.
no. of sealers REF 1089 no. 2 REF 1089.1 no. 4

Package insert (REF 78109-IFU) **–** The present document.

Symbol information sheet (REF INSYS01) – List of the symbols.

Note - All the materials of human origin have been controlled and certified by the supplier to be negative for HBsAg, HCV Ab and HIV Ab.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Micropipettes of 20, 100, 300 and 1000 µl with disposable tips.
2. Vortex mixer and adsorbent papers.
3. Distilled water.
4. Timer.
5. Incubator set at 37 ± 1 °C (dry or moist heat).
6. Automatic or manual microplate washer able to aspirate and dispense volumes of 300 - 400 µl.
7. Photometric microplate reader linear up to at least 2 OD and supplied with filters of 450 nm and 620 - 630 nm.

PREPARATION OF REAGENTS
Washing Solution - The concentrated solution to be diluted 25x with distilled water before use.

PREPARATION OF REAGENTS OF KITS 1089/1089.X
Chromogen/Substrate - About 5 minutes before use, mix 1 volume of Chromogen with 1 volume of Substrate, in a disposable plastic container, according to needs. *This solution is stable for 4 hours at room temperature protected from light.*

PREPARATION OF REAGENTS OF KITS 1089/1089.X
Conjugate - Dilute the concentrated Conjugate 1:20 with the Conjugate Diluent. Mix on vortex before use. *The diluted Conjugate is stable for 1 week at 2 – 8 °C, when stored in a sterile disposable container.*

SPECIMEN COLLECTION AND STORAGE
Either fresh sera or plasma (EDTA, Heparin, Citrate) can be used for the assay. If not used immediately, they can be stored at 2 - 8 °C for 1 week. In case of longer storage freeze them at – 20 ° C. Samples should be clear. If the samples are turbid, could be contamined by microorganism, insofar it recommends to centrifugate them at 2000 rpm x 20 minutes at room temperature or filtrate on 0.22 µm filters.
The samples that, after the above said procedure, did not became clear, can not be used.

WASHING INSTRUCTIONS
A good washing procedure is essential to get correct and reliable analytical results.
In case of manual washing, it is suggested to carry out 5 cycles, first dispensing and then aspirating 300 µl/well per cycle.
Usually 5 cycles of automatic washing of 300 µl/well per cycle are sufficient to remove false positives and high background values.
It is suggested to use an Elisa automatic microplate washer, qualified and properly serviced. It is highly recommended to calibrate the washing system on the kit itself so to match the declared analytical performances.
Potentially infective wastes from microplate washing have to be inactivated with Na-hypochlorite at 2.5% final concentration for 30 minutes. All these materials have to be discarded according to the law as potentially infective wastes.

ASSAY PROCEDURE
At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Do not dilute Controls as they are ready to use.
1 – Leave the A1 well empty for blanking operations. Dilute all the Samples 1 : 101 with the Sample Diluent (for example, 500 µl of Sample Diluent + 5 µl of Sample) and mix on vortex. Do not dilute Controls as they are ready-to-use.
Dispense 50 µl of Neutralizing Reagent in all the wells, except in A1 used for blanking and in the wells used for Controls (B1+C1+D1+E1).
Then dispense Controls and samples into wells according to the following table:

Position	*Controls/Samples*
A1	Blank
B1+C1+D1	100 µl of Negative Control
E1	100 µl of Positive Control
F1.....H12	50µl of Diluted Samples

Cover the microplate with the plate sealer and incubate strips for 60 minutes at 37 °C.

2 – Peel off the plate sealer and wash the microplate according to instructions. In the meantime dilute the quantity of concentrated Conjugate.

3 – Add 100 µl of diluted Conjugate to all the wells, but A1.

4 – Incubate the microplate sealed for 60 minutes at 37 °C.

5 – Peel off the plate sealer and wash the microplate according to instructions. In the meantime dilute the necessary quantity of Chromogen/ Substrate solution (1089/1089.X).

6 – Add 100 µl of the Chromogen/Substrate solution to all the wells, A1 included.

7 - Incubate the microplate for 20 minutes at room temperature, protected from light.

8 – Stop the enzymatic reaction by adding 100 µl of Stop Solution to all the wells, A1 included. Read the microplate at 450 nm and 620 - 630 nm blanking the instrument on A1 well.
Note - Read the microplate within 30 minutes after the dispensing of the Stop Solution.

ASSAY SCHEME
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Position	*Controls/Samples*
A1	Blank
B1+C1+D1	100 µl of Negative Control
E1	100 µl of Positive Control

Reagents	*Blank (A1)*	*Controls*	*Samples*
Neutralizing Reagent	**-**	**-**	**50 µl**
Controls	**-**	**100 µl**	**-**
Diluted Samples	**-**	**-**	**50µl**
Cover with the sealer and incubate for 60 minutes at 37 °C			
Peel off the sealer and wash 5 cycles with 300 µl/well per cycle Dilute the quantity of concentrated Conjugate			
Conjugate	**-**	**100 µl**	**100 µl**
Cover with the sealer and incubate for 60 minutes at 37 °C			
Peel off the sealer and wash 5 cycles with 300 µl/well per cycle Dilute the necessary quantity of Chromogen/Substrate solution (1089 & 1089.X)			
Chromogen/Substrate	**100 µl**	**100 µl**	**100 µl**
Incubate for 20 minutes at room temperature in the dark			
Stop Solution	**100 µl**	**100 µl**	**100 µl**
Blank the reader on A1 well. Read at 620 - 630 nm for measuring the Microplate background, then at 450 nm.			
The Microplate reading must be done within 30 minutes from the dispensing of the Stop Solution,			

RESULT INTERPRETATION
Validity of the Assay
The assay is considered valid if:
1. The OD 450 nm of the A1 blank well is < 0.100. Higher values are index of Chromogen/Substrate contamination.
2. After blanking on A1, the OD 450 nm mean value of the Negative Control (NC) is < 0.200. Abnormal values may be
   observed when the washing instrument does not work correctly or the washing procedure has not been adapted to the assay as described in the proper section.
3. The OD 450 nm value of the Positive Control (PC) is > 0.500. Lower values can be result when the storage temperature was not optimal or with an incorrect operational procedure.

In case that the above data do not match the correct values, before repeating the test check carefully the expiration date of the kit, the performances of the instruments used for the assay and the procedure of distribution of Controls and samples.

Calculation of Results
If the validity of the assay is confirmed, calculate the Cut-off (Co) value through the following formula:

Cut-off = NC mean + 0.100

Example of calculation
Negative Control (NC) mean OD 450 nm 0.050
Positive Control (PC) OD 450 nm 1.200
Cut-off = NC + 0.100 = 0.150
Sample # 1 OD 450 nm = 0.080 negative
Sample # 2 OD 450 nm = 1.158 positive
Samples with an OD 450 nm value lower than the Cut-off are classified as negative for anti-CoV19 IgM antibodies.
Samples with an OD 450 nm value higher than the Cut-off are classified as positive for anti-CoV19 IgM antibodies.

LIMITATIONS OF THE PROCEDURE
Highly lipemic, icteric, hemolysed samples or repeatedly defrosted samples and therefore subject to contamination, should not be used as they can give false results in the assay.
Some false positives have been reported by Literature for “sandwich” IgM assays due to very high concentrations of interferents.

PROCEDURE AUTOMATION
This procedure can be used with an automated device under the customer’s responsibility provided they validate the results with an adequate method. For more information, please contact the automated device manufacturer.

PERFORMANCE CHARACTERISTICS
Sensitivity -The sensitivity of the assay has been calculated on a panel of positive samples by comparing with a FDA approved kit on the market. The test shows a sensitivity: 100 %.

Specificity - It has been calculated on panels of negative samples, pre-classified with an FDA approved kit present on the market. The assay shows a specificity ≥ 99,5 % on plasma and sera.

Reproducibility - A set of negative and positive samples was repeatedly tested on different days in order to determine the statistical values of reproducibility for evaluating the inter-assay variance. The mean value of CV% for OD 450 nm higher than 0.500 (Positive Samples) is lower than 20 %, the mean value of CV% for OD 450 nm lower than 0.200 (Negative Samples) is lower than 30 %.

Repeatability – A set of evaluation intra-assay of negative donor specimens and positive specimens, gives a CV% value ≤ 30% for the negative, ≤ 20% for the positive.

PRECAUTIONS IN USE
The use of the laboratory reagents according to Good Laboratory Practice (GLP) is recommended.

WASTE MANAGEMENT
Please refer to local legal requirements.

REFERENCES

1. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding.Lancet (London, England) 2020 ;395(10224):565–74. [PMC free article] [PubMed][Google Scholar]
2. Zhang L, Shen FM, Chen F, Lin Z. Origin and evolution of the 2019 novel coronavirus.Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2020 [PMC free article] [PubMed] [Google Scholar]
3. Perlman S. Another Decade, Another Coronavirus. New England Journal of Medicine.2020;382(8):760–2. [PMC free article] [PubMed] [Google Scholar]
4. Phelan AL, Katz R, Gostin LO. The Novel Coronavirus Originating in Wuhan, China: Challenges for Global Health Governance. Jama. 2020 [PubMed] [Google Scholar]
5. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. The Lancet Infectious diseases. 2020 [PMC free article] [PubMed] [Google Scholar]
6. Kickbusch I, Leung G. Response to the emerging novel coronavirus outbreak. BMJ .2020:368. [PubMed] [Google Scholar]





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