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ICMR-NIV Anti-SARS CoV-2 Human IgG ELISA

MaxLISA Covid Kawach™

INTENDED APPLICATION

NIV SARS CoV-2 Human IgG ELISA kit is intended for qualitative detection of IgG antibodies in serum/plasma of patients presenting clinical signs and symptoms consistent with SARS CoV-2 infection.

Caution: The laboratory results alone should not form the basis of medical report for individual patient. The clinical history and any other test performed must be taken into account. The presumptive diagnosis by SARS CoV-2 Human IgG ELISA may be confirmed by Neutralization assay.

INTRODUCTION

Novel corona virus infection SARS CoV-2 [COVID-19] has spread to more than 203 countries of various regions including Africa, America, Europe, South East Asia and Western Pacific. The WHO had declared COVID-19 as the global public health emergency and subsequently as pandemic because of its worldwide spread. It is now one of the top-priority pathogens to be dealt with, because of high transmissibility, severe illness and associated mortality, wide geographical spread, lack of control measures with knowledge gaps in veterinary and human epidemiology, immunity and pathogenesis. The quick detection of cases and isolating them has become critical to contain it. Whereas molecular diagnostic tests were rapidly developed, serologic assays are still lacking, yet urgently needed. Validated serologic assays are important for sero-surveillance and sero-epidemiological studies.

PRINCIPLE OF ASSAY:

IgG antibodies from serum / plasma of human will bind to the SARS CoV-2 virus whole cell antigen coated on to the Microtitre plate (ELISA wells). In the next step, anti Human IgG HRP binds to captured human IgG antibodies. Subsequently, chromogenic substrate (TMB/H2O2) is added, the reaction is stopped by 1N H2SO4. The intensity of color / optical density is measured at 450 nm. The kit is for *in- vitro* use for monitoring anti-SARS CoV-2 antibodies in human only.

REAGENTS PROVIDED IN EACH KIT:

Table-1: Kit components

Sno.	Kit Components	Quantity	Working dilution
1.	Coated Micro-well strips		Not applicable
2.	Conjugate	12ml	Ready to use (Light sensitive)
3.	Positive Control (PC)	10µl	1:100
4.	Negative Control (NC)	10µl	1:100
5.	Wash buffer (20X)	60ml	1X
6.	Sample diluent powder	3gm	
7.	Sample diluent	20ml	Ready to use after addition of Sample diluent powder
8.	TMB	12ml	Ready to use
9.	Stop solution	12ml	Ready to use

Antigen coated strips - Ready to use

(Twelve Strips with eight wells each)

Polystyrene wells are coated with SARS COV-2 antigen. This kit is stable at 2-8°C if protected from moisture.

Sample diluent for Anti-SARS CoV-2 IgG- One bottle (15 ml / kit)

3gm skimmed milk to be dissolved in 60ml of 1x PBST (WASH BUFFER). Can be Stored at 4°C for 1 week.

SARS CoV-2 Human IgG Positive and Negative Controls-

Human serum positive and negative controls to be used in 1: 100 dilutions. Dilution to be made in diluent (5% skimmed milk).

Wash buffer concentrate (20X)-1 Bottle (60 ml)

Phosphate buffered saline with surfactant and antibiotics. If the Wash Buffer Concentrate shows crystallization, warm the bottle at 37°C until crystallization disappears. Before use, dilute wash buffer concentrate 1:19 (1 part of buffer concentrate + 19 parts of high grade distilled water) to prepare 1X Wash Buffer.

a. Conjugate – Ready to use. Protect from light

b.Liquid TMB (Tetramethylbenzidine Dihydrochloride). **substrate** - 1 bottle (12ml) Protect from light. Ready to use.

c. Stop solution- 1 bottle (12ml) Wear protective gloves, mask and eye glasses while handling stop solution. Ready to use.

STORAGE CONDITIONS:

It is essential that all reagents/material is stored at 2-8°C. Do not freeze any of the reagents. Do not bring / equilibrate any of the regents to room temperature even on the day of performing the test. Protect the ELISA wells from moisture. The unused strips should be placed safely in pouch.

ADDITIONAL MATERIAL REQUIRED BUT NOT SUPPLIED

1. Biosafety Cabinet
2. Microplate washer
3. Microplate reader with 450 nm filter
4. Incubator (37°C), humidified without Co_2
5. Adjustable micropipette (stepper multichannel) with disposable tips (2 µl to 500 µl)
6. High grade class distilled water (2.5 Liters).
7. Deep well (2 ml) plates
8. Bread Box (A plastic box with tight cover), Tissue paper , Aluminum foil, Timer, Vortex

PRECAUTIONS FOR CONSISTENT / ACCURATE RESULTS

Read the kit insert thoroughly before carrying out the test.

- Do not use kit reagents after expiry.
- Do not use reagents from a different batch number.
- Do not freeze any of the reagents.
- On the day of performing a test, do not bring the reagents to room temperature.
- Avoid exposure of kit reagents to direct sunlight or higher temperature than recommended storage temperature.
- Use protective clothing, hand gloves, and glasses while performing the assay.
- Avoid mouth pipetting.
- If any of the reagents show precipitation / mix it by repeated pipetting.
- Use clean, sterile, low protein binding tips and calibrated micropipettes.
- Use bench / disposable absorbent paper (Filter paper) to cover the working table.
- Avoid Microbial contamination of any of the reagents or cross contamination of different reagents.
- Use the kit for testing Serum/plasma only. The kit is not optimized for testing other body fluids or whole blood. Serum with bacterial / fungal contamination or hemolysed may give invalid results.
- Do not use freeze-thawed serum samples
- Incomplete washing may adversely affect the results of the assay.
- Poor quality distilled water (for dilution of wash buffer concentrate) can lead to erroneous results.
- After washing the wells, add the next reagent immediately as per the protocol. Do not allow the wells to dry.

POTENTIAL BIOHAZARD MATERIAL

The antigen coated to the wells has been inactivated as per the WHO guidelines. The virus inactivation was confirmed before use. But since the sera being tested for presence of SARS CoV-2 specific IgG antibodies are from unknown human these could be infectious although infection of laboratory worker due to handling of human sera has still not been documented. Handle all samples and the unused kit reagents as infectious. Follow standard guidelines for handling and disposing off. Biosafety norms of your institute for handling animal samples must strictly be followed.

TEST PROTOCOL

Thoroughly mix all the reagents using the micropipette tips before addition. The test procedure must be followed meticulously. Don't bring any of the kit reagents to room temperature before commencing the test. All reagents are stable at 2-8^{0}C and for in vitro use only.

Preparation of reagents before performing test

a. Dilute wash buffer concentrate to 1 x (Mix one part of wash buffer concentrate with19 parts of high grade class distilled water. The unused diluted Wash Buffer can be stored in a refrigerator until it shows microbial growth.

b. Prepare sample diluents buffer by adding 1gm of sample diluent powder in 20ml of sample dilution buffer

c. Select the samples to be assayed. Write down the protocol on ELISA sheet.

d. Inactivate the samples at 56^{0}C for 30 min

e. Dilute the samples to be tested prior to initiating the test proper (5µl of sample + 495µl of sample diluents buffer)

Brief ELISA Protocol

1. Wash the required number of coated strips, three times with 1X Wash Buffer.
2. After washing, invert the plate and tap on dry absorbent paper.
3. Add 100 µl of Positive Control in A1 and B1
4. Add 100 µl of Negative Control in C1 and D1
5. Add 100 µl of diluted sample(1:100) in respective wells ((E1,F1,G1,H1;A2, B2…..)
6. Place the plate in a container with a lid that contains absorbent material, wet with water (such as a paper towel or cotton balls) to create humidity chamber.
7. Incubate at 37^{0}C for 60 minutes.
8. Wash the wells 5 times with 1X Wash Buffer. After washing, invert the plate and tap on dry absorbent paper.
9. Add 100 µl of conjugate each well and place the plate in a humidity chamber.
10. Incubate at 37^{0}C for 60 minutes.
11. Repeat Step no. 8.
12. Add 100 µl of Substrate into each well and place the plate in a humidity chamber.
13. Incubate at Room temperature for 10 minutes in dark.
14. The reaction can be stopped by adding 100 µl Stop solution.
15. Read on plate reader at 450 nm.

QUALITY CONTROL:

Each kit contains one vial of **"Positive** control" and one vial of **"Negative** control". These work as markers of kit performance.

P/N ratio of **Positive** control is defined as ratio of OD value of **Positive** control divided by average OD of **Negative** control.

$$\textbf{P/N ratio} = \frac{\textbf{Average OD value of Positive control}}{\textbf{Average OD value of Negative control}}$$

The test is considered to be valid if:

P/N ratio of Positive control is greater than 1.5

Cutoff calculations = Av. NC + 0.2

$$\textbf{P/N ratio OF SAMPELS} = \frac{\textbf{O.D value of sample}}{\textbf{Average OD value of Negative control}}$$

Interpretation of the results:

1. For an unknown sample (test sample) if O.D value > Cutoff value and P/N ration more than 1.5, sample should be considered as "Positive".
2. For an unknown sample (test sample) if O.D value < Cutoff value and P/N ration less than 1.5, sample should be considered as "Negative".

SPECIFIC PERFORMANCE CHARACTERISTICS

1. Specificity: 97.9%
2. Sensitivity: 92.3%

TEST LIMITATIONS:

a. **Epidemiology of SARS CoV-2** in the geographical region should be considered critical for interpretation of the results.

b. The performance of the assay has not been optimized for visual determination (OD).

TROUBLE SHOOTING GUIDE

No color development

Possible cause	Corrective action
Have you omitted any step?	Follow the protocol meticulously
Reagents were added in wrong order.	Follow the protocol meticulously
Strips not washed before sample addition	Wash plate three times with wash buffer before sample addition
Conjugate has lost activity	In a glass tube, add 20µl of conjugate and 20 µl of liquid TMB substrate (Ready to use) and check for color development.

Low OD value of "Positive control"

Possible cause	Corrective action
Stop Solution was added before 10 mins. Reaction terminated before 10mins.	Follow the protocol meticulously
OD taken at incorrect wavelength	Read OD values at 450 nm

High OD values of "Negative control"

Possible cause	Corrective action
Plate terminated after 10 mins	Follow the protocol meticulously
Same pipette used for Positive and Negative controls	Change micropipette tips while addition of Negative / Positive control
Nonspecific attachment / binding of other reagent	If plates get scratches/aberrations during washing, nonspecific proteins may bind while addition of next step

High OD reading in most of the wells (High background)

Possible cause	Corrective action
Liquid substrate not properly protected from light.	Incubate the plate in dark after addition of substrate
Contamination of liquid Substrate	Check OD value of substrate blank.
Insufficient washing	Follow wash protocol meticulously
Poor quality of water used for diluting wash buffer concentrate	Glass distilled water is preferred

Poor reproducibility of test

Possible cause	Corrective action
Dispensing errors	Calibrate micropipettes. Check other dispensing equipment
Improper washing	Check all eight ports/manifold for uniform flow of wash buffer. If there are blockages, clean the ports.

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