COVID KAWACH IgG MICROLISA

Microwell ELISA Test for the Qualitative Detection of Covid-19 (SARS-COV-2) IgG antibodies in Human Serum/Plasma

1. 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.

2. INTENDED USE

Covid Kawach IgG Microlisa 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.

3. PRINCIPLE

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 IgG antibodies in human only.

4. DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2016.

	Manufactured By	IVD	*In vitro* diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number Batch Number	2°C 8°C	Temperature Limitation
	Manufacturing Date		Caution See instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged		Keep away from sunlight
	Keep Dry		

5. KIT PRESENTATION

- 96 Test Pack

6. KIT & ITS COMPONENTS

Components	Qty.	Description
Microwells	96 Wells	Microwells coated with SARS CoV-2 virus whole cell antigen packed in a pouch provided with desiccant.
Sample Diluent	62 ml	Buffer containing protein stabilizers and antimicrobial agents as preservative.
Enzyme Conjugate	12 ml	Anti-Human IgG labelled with horseradish peroxidase with protein stabilizers. (Ready to use)
Sample Diluent Powder	3x1 gm.	Skimmed milk powder
Wash Buffer Concentrate (20X)	60 ml	PBS with surfactant. Dilute 1:20 with distilled water before use.
TMB Substrate	10 ml	TMB solution
TMB Diluent	10 ml	Buffer solution containing H_2O_2 with preservative
Control —	0.05 ml	Normal human serum negative for Covid Antibodies. To be diluted 1:100.
Control +	0.05 ml	Non infections, Positive serum for Covid IgG antibodies. To be diluted 1:100.
Stop Solution	12 ml	Ready to use, 1N sulfuric acid
Plate Sealers	4 nos.	Adhesive sheets to cover the microwells during incubation.

7. STORAGE AND STABILITY

Store the kit & its components at 2-8°C Expiry date on the kit indicates the date beyond which kit should not be used. Do not freeze any of the kit components.

8. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- Micropipettes and microtips
- Elisa reader
- Distilled or deionized water
- Graduated Cylinders, for reagent dilution
- Paper towels or absorbent tissue
- Biosafety Cabinet
- Plastic box with tight cover
- Timer
- Elisa washer
- Incubator 37°C, humidified without CO_2
- Disinfectant solution
- Disposable gloves
- Vortex

9. SPECIMEN COLLECTION & HANDLING

1. Human serum or plasma samples should be used for the test. While preparing serum samples, remove the serum form the clot as soon as possible to avoid hemolysis. Fresh serum/plasma samples are preferred.
2. Specimens should be free of microbial contamination and may be stored at 2-8°C for one week, or frozen at -20°C or lower.
3. Do not use freeze thawed serum samples.
4. **Inactivate the samples at 56°C for 30 minutes before use.**
5. Hemolyzed and Icteric hyperlipemic samples may give erroneous results.

10. CAUTION

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.

1. The use of Disposable Gloves, glasses and proper portecting clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Do not pipette by mouth.
5. Tests are for in vitro diagnostic use only and should be run by competent person only.
6. All the samples to be tested should be handled as though capable of transmitting infection.
7. All materials used in the assay and samples should be decontaminated in suitable disinfectant solution for 30-60 min. before disposal. They should be disposed off in accordance with established biosafety guidelines for handling & disposal of potentially infective material.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. Wash hands thoroughly with soap or any suitable detergent, after the use of the

kit. In case of needle prick or other skin puncture or wounds, wash the hands with excess of water and soap.

10. Controls contain Sodium Azide as a preservative. If these materials are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds.
11. ELISA Reader & micropipettes used in testing should be calibrated at regular interval to ensure accurate results.

11. PRECAUTIONS FOR USE

Optimal assay performance requires strict adherence to the assay procedure described in the manual.

1. Do not use kit components beyond the expiration date, which is printed on the kit.
2. Avoid microbial contamination of reagents. The use of sterile disposable tips is recommended while removing aliquots from reagent bottles.
3. Stop solution contains sulfuric acid. If sulfuric acid comes in contact with the skin, wash thoroughly with water. In case of contact with eyes, flush with excess of water.
4. Take care while preparing working substrate solution as vials of TMB substrate & diluent are of same size.
5. Prepare working substrate solution just 10 minutes prior to adding in the wells.
6. If any of the reagent show precipitation, mix it by repeated pipetting before use.
7. Use separate tips for TMB substrate and TMB diluent.
8. Do not allow microwells to dry once the assay has started.
9. Ensure that the microwell strips are levelled in the strip holder. Before reading, wipe the bottom of the microwell strips carefully with soft, absorbent tissue to remove any moisture.
10. Distilled or deionised water must be used for wash buffer preparation.
11. Bring all the reagents to room temperature (20-30^{0}C) before use.
12. Do not combine reagents form different batches, as they are optimized for individual batch to give best results.
13. Due to interchange of caps the reagents may get contaminated. Care should be taken while handling the reagents to avoid contamination of any sort.
14. Run negative and positive controls in each assay.
15. Use freshly collected, clean serum/ plasma samples for assay. Try to avoid Haemolyzed turbid, lipemic serum or plasma samples.
16. Use a separate tip for each sample and then discard it as biohazardous waste.
17. Thorough washing of the wells is critical to the performance of the assay.
18. Avoid strong light exposure during the assay.

12. PREPARATION OF REAGENTS

Prepare the following reagents before or during assay procedures. Reagents and samples should be at room temperature (20-30^{0}C) before beginning the assay and can remain at room temperature during testing. Return reagents to 2-8^{0}C after use. All containers used for preparation of reagents must be cleaned throughly and rinsed with distilled or deionized water. Prewarm the incubator to 37^{0}C.

i) SARS CoV-2 virus whole cell antigen coated strip

Bring foil pack to room temperature (20-30^{0}C) before opening to prevent condensation on the microwell strips.

a. Break-off the required number of strips needed for the assay and place in the well holder. Take the strip holder with the required number of strips, taking into account that two negative & two positive control should be included in the run while opening the fresh kit.

b. **Unused wells should be stored at 2-8^{0}C, with dessicant in an aluminium pouch with clamp & rod.**

Caution: Handle microwell strip with care. Do not touch the bottom exterior surface of the wells.

ii) Preparation of Working Wash Buffer:

a) Check the buffer concentrate for the presence of salt crystals. If crystals are present in the solution, resolubilize by warming at 37^{0}C until all crystals dissolve.

b) Prepare at least 40 ml (2 ml concentrated buffer with 38 ml distilled water) of buffer for each strip used. Mix well before use.

c) Working Wash buffer can be stored in a refrigarator at 2-8°C until it shows microbial growth.

iii) Preparation of Working Sample Diluent:

The working sample diluent is used for dilution of sample and controls. For the preparation of the working sample diluent, take 1 Sample Diluent Powder pouch and transfer the complete content of the pouch to 20 ml of sample diluent. Mix the working sample diluent properly using vortex before use.

The working sample diluent is stable for 1 week at 2-8^{0}C.

iv) Sample & Control Preparation:

TUBE DILUTION: Mark the tubes carefully for the proper identification of the samples, negative control and positive control. Dilute the controls and serum samples to be tested, with sample diluent 1:100 in separate tubes (495 μl. sample diluent + 5 μl samples / controls). Use a separate tip for each sample / control and then discard as biohazardous waste.

v) Preparation of working substrate solution :

Mix TMB substrate and TMB Diluent in 1:1 ratio to prepare working substrate.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
TMB Substrate (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
TMB Diluent (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0

Do not store working substrate. Prepare a fresh dilution for each assay in a clean plastic/glass vessel. Determine the quantity of working substrate solution to be prepared from table. Mix solution thoroughly before use. Discard unused solution. A deep blue color present in the substrate solution indicates that the solution has been contaminated and must be discarded.

13. PROCEDURAL NOTES:

1. Material should not be used after the expiry date shown on the labels. Components and test specimen should be at room temperature (20-30^{0}C) before testing begins. Return the reagents to 2-8^{0}C after use.
2. All reagents must be mixed well before use.
3. To avoid contamination, do not touch the top or bottom of strips or edge of wells.
4. All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.
5. Prevent evaporation during sample incubation by covering the strips with sealer; remove sealer before washing.
6. Routine maintenance of wash system is strongly recommended to prevent carry over from highly reactive specimens to non reactive specimens.
7. After washing the wells, add the next reaget immediately as per the test protocol. Do not allow well to dry.

14. TEST PROCEDURE

Once the assay has started, complete the procedure without interruption. All the reagents should be dispensed in the centre of the well and the tip of the pipette should not touch the wall of the microwell.

Fit the stripholder with the required number of Covid Antigen coated strips. The sequence of the procedure must be carefully followed. Arrange the assay control wells in a horizontal or vertical configuration. Configuration is dependent upon reader software.

1. Wash the required no. of coated strips 3 times with working wash buffer (1x). After washing, invert the plate and tap on dry absorbent paper.
2. Add 100μl diluted Negative Control in each well no. A-1 & B-1 respectively.
3. Add 100μl diluted Positive Control in C-1 & D-1 wells.
4. Add 100 μl diluted sample in each well starting from E-1.
5. Apply cover seal and place the plate in a container with a lid that contains absorbent material, wet with water (such as paper towel or cotton balls) to create humidity chamber.
6. Incubate at 37^{0}C $\pm$ 1^{0}C for 60 min. $\pm$ 1 minute.
7. While the samples are incubating, prepare working Wash Solution as specified in preparation of reagents.
8. Take out the plate from the incubator after the incubation time is over and, wash the wells 5 times with working Wash Solution. After washing, invert the plate and tap on dry absorbent paper.
9. Add 100 μl of Enzyme Conjugate Solution in each well.

10. Apply cover seal and incubate at 37°C ± 1°C for 60 min ± 1 minute in a humidity chamber.
11. Aspirate and wash as described in step no.8.
12. Add 100 µl of working substrate solution in each well.
13. Incubate at room temperature (20-30°C) for 10 minutes in dark.
14. Add 100 µl of Stop Solution.
15. Read absorbance at 450 nm in ELISA READER.

SUMMARY OF PROCEDURE		
Wash		3 Cycles
Dilute Sample & controls in working sample diluent		495 µl working sample diluent + 5 µl sample / controls
Add samples & controls		100 µl
Cover the plate & incubate		60 mins. at 37°C
Wash		5 Cycles
Add Enzyme Conjugate		100 µl
Cover the plate & incubate		60 mins. at 37°C
Wash		5 Cycles
Prepare Working Substrate		No of Strips: 1 2 3 4 5 6 7 8 9 10 11 12 TMB Substrate (ml): 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 TMB Diluent (ml.): 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0
Add Substrate		100 µl
Incubate in dark		10 mins. at Room Temp.
Add Stop Solution		100 µl
Read Results		450 nm

15. 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 O.D. value of **Positive** control divided by O.D. of average O.D. of **Negative** control

$$\text{P/N ratio} = \frac{\text{O.D. value of Positive control}}{\text{Average O.D. value of Negative control}}$$

The test is considered to be valid if: P/N ratio of Positive control is greater than 1.5

16. CALCULATION OF RESULTS

a. Cut off value = NCx + 0.2

b. Calculation of P/N ratio of samples : Calculate P/N ratio of samples as follows:

$$\text{P/N ratio of samples} = \frac{\text{Sample O.D.}}{\text{Average O.D. of negative control}}$$

17. INTERPRETATION OF RESULTS

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

18. PERFORMANCE CHARACTERISTICS

The performance of 3 batches of Covid Kawach IgG Microlisa has been validated by ICMR, NIV, Pune. The results obtained are as follows:

Sensitivity : 96.33% **Specificity : 100%**

Precision : Within-run and between-run precisions have been determined by testing 10 replicates of five specimens : two negative and three weak covid IgG positive. The C.V.(%) of negative and weak positive values were within 10%.

19. LIMITATION OF THE TEST

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).

20. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in vitro diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacture's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed.

The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

21. REFERENCES

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22. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. No colour or light colour developed at the end of assay	a) Any one reagent has been added in wrong sequence.	Follow the procedure meticulously & repeat assay.
	b) Inactivated conjugate, improper storage	In a glass tube, add 20 µl of enzyme conjugate and 20 µl of working TMB Substrate and check for yellow color development
	c) Microplate inactivated, due to improper storage	Keep unused strips in sealable poly pouch, very well closed with the dessicant pouch inside
	d) Inactivated substrate, improper storage or preparation	Use freshly prepared substrate solution. Recheck procedure, repeat assay
	e) Omission of any step in test procedure	Follow the procedure meticulously & repeat assay.
	f) Strips not washed before sample addition	Wash plate 3 times with working wash buffer before sample addition
2. High O.D. value of Negative control	a) Plate not stopped after 10 minutes of additing stop solution	Follow the procedure meticulously & repeat assay.
	b) Sample microtip used for Positive and negative controls	Change micropipette tips while addition of negative/ positive control

PROBLEM	POSSIBLE CAUSE	SOLUTION
	c) Nonspecific attachment/ binding of other reagent	If plates get scratches/ aberrations during washing, non specifi proteins may bind while addition of next step.
3. Too much colour in all wells of the plate (high background)	a) Contaminated substrate	Check substrate (TMB Diluent) it should be colourless. If blue in colour then discard and use clean disposable container.
	b) Contaminated washing solution (1X). Poor quality of water used for diluting wash buffer conc.	Check the container and quality of water used for dilution. Use of glass distilled water is preferred.
	c) Over incubation of substrate and delay in addition of stop solution.	Follow the procedure meticulously.
	d) Insufficient washing. i) Washing not consistent	Check wash device, fill the well close to the top.
	ii) Filling volume not sufficient.	After washing, blot the microwells on absorbent
	iii) Insufficient no. of wash cycles.	tissue. Follow wash protocol meticulously
	iv) Contaminated wash device	
	e) Use of wash solution from other manufacturer.	Use only Covid Kawach IgG Microlisa wash solution.
	f) Working substrate not protected from light	Incubate the plate in dark after addition of substrate.
4. Poor reproducibility	a) Washing problems.	Check all 8 ports/ manifold for uniform flow of wash buffer. If there are blockage, clen the ports.
	b) Uncalibrated pipettes or tips not well fitted, improper pipetting/ dispensing.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	c) Interference in optical pathway due to Air bubbles.	Clean or dry the bottom of microwells, check for bubbles and repeat the readings.
5. False Positive	Beside 3a, b, c, d, e incorrect interpretation and calculation of final results	Check the calculation part given in the insert and correctly interpret.
6. False Negative/ low O.D. of Positive control & positive sample	a) Inadequate addition of substrate/conjugate solution.	Recheck the test procedure and reagent volume.
	b) Kit expired, reagent of different kit used.	Check the expiry of the kit before use.
	c) White particles in working substrate solution.	Discard the substrate and prepare the working substrate again in fresh tube.
	d) Uncalibrated pipettes, improper pipetting.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	e) Deterioration of Enzyme conjugate	Check storage of Enzyme conjugate. It shall be stored at 2-8°C.
	f) Stop solution is added before 10 minutes. Reaction terminated before 10 minutes.	Follow the test procedure meticulously.
	g) O.D. taken at incorrect wavelength.	Read O.D. values at 450 nm only.

TECHNOLOGY TRANSFER FROM:

ICMR INDIAN COUNCIL OF MEDICAL RESEARCH Serving the nation since 1911 | NIV NATIONAL INSTITUTE OF VIROLOGY

in vitro diagnostic Reagent, not for medicinal use

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