GENLISA™ Human Anti-SARS-CoV-2 (Covid-19) IgM Antibody to spike protein Qualitative ELISA

REF : KBVH015-6

Ver 1.1

RUO

Enzyme Immunoassay for the Qualitative screening of IgM Antibodies to Human SARS-CoV-2 (Covid-19) spike protein in human serum and plasma

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBVH015-6 **Σ 96 tests**

KRISHGEN BioSystems For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005
For Asia/India Customers: tel +91(22)-49198700
Email: sales@krishgen.com | http://www.krishgen.com

Introduction:

The GENLISA™ ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies are used in this kit.

Intended Use:

The GENLISA™ Human Anti-SARS-CoV-2 (Covid-19) IgM antibody to spike protein qualitative ELISA kit is used as an analytical tool for screening for IgM antibodies to Human SARS-CoV-2 (Covid-19) in human serum and plasma.

Principle:

The method employs sandwich ELISA technique. Human SARS-CoV-2 protein is pre-coated onto microwells. Samples and Controls are pipetted into microwells and IgM Antibodies to human SARS-CoV-2 (Covid-19) present in the sample are bound by the protein antigen. After incubation the wells are washed and followed by HRP-conjugated Detection IgM antibody is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of IgM Anti-Human SARS-CoV-2 (Covid-19) in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Recombinant SARS-CoV-2 (Covid-19) spike protein Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Anti-Human SARS-CoV-2 (Covid-19) spike protein Positive Control (concentrated, lyophilized) - 2 vials
3. SARS-CoV-2 (Covid-19) Antibody:HRP Conjugate - 12 ml
4. Control Diluent - 10 ml
5. (1X) Assay Diluent – 2*50 ml
6. (20X) Wash Buffer - 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction Manual
10. Plate Sealer

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Store kit components at 2-8°C.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided.

Serum and Plasma Samples should be diluted 1:1000 (v/v) for optimal recovery, (for example 1 ul sample + 999 ul assay diluent) prior to assay. In cases where matrix interferences is under or over observed, the samples may be diluted with Assay Diluent accordingly.

The samples may be kept at 2-8°C for up to three days. For long-term storage please store at -20°C.

Note: *Grossly hemolyzed samples are not suitable for use in this assay*

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room Temperature before use.
3. To make **Wash Buffer (1X)**; dilute **25 ml** of **20X Wash Buffer** in **475 ml of DI water**.
4. **Positive Control**: Reconstitute the concentrated Positive Control lyophilized vial with 1 ml of Control Diluent. Keep the vial for 15 mins with gentle agitation before using. Use Control Diluent as **Negative Control**.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Anti-Human SARS-CoV-2 (Covid-19) IgM. High Dose Hook Effect is due to excess of antibody for very high concentrations of Anti-Human SARS-CoV-2 (Covid-19) IgM present in the sample.
3. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent.
4. Anti-Human SARS-CoV-2 (Covid-19) IgM concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
5. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Anti-Human SARS-CoV-2 (Covid-19).
6. It is recommended that all Controls and Samples be assayed in duplicates.
7. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
8. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
9. The plates should be read within 30 minutes after adding the Stop Solution.
10. Make a work list in order to identify the location of Controls and Samples.

Assay Procedure:

1. Pipette **100 ul** of **reconstituted Positive Control, Negative Control** and **diluted Samples** to the respective wells and incubate at Room Temperature (18-25°C) for 1 hour.

2. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

3. Add **100 ul** of **Anti-Human IgM:HRP Conjugate** to each well.

4. Seal plate and incubate for 1 hour at Room Temperature (18-25°C).
5. Wash plate 4 times with **Wash Buffer (1X)** as in step 2.
6. Pipette **100 ul** of **TMB Substrate solution**.
7. Incubate in the dark for 15 minutes at Room Temperature.
8. Stop reaction by adding **100 ul** of **Stop Solution** to each well.
9. Read absorbance at 450 nm within 30 minutes of stopping reaction.

Interpretation of the Results:

Calculation for Cut off Values
Read the sample and negative control wells on microtitre plate reader at 450nm. The OD (Optical Density) of NC (Negative Control) in duplicate should be used for calculating the mean and standard deviation. This is the $Negative_{mean}$.

The cut-off for Positives is equal to a value greater than ($Negative_{mean}$ + 3*Standard Deviation).

Formula:
Positive Sample Value = OD > (*Negative$_{mean}$ *+ 3*SD)

Typical example –

Sample Type	*Absorbance #1*	*Absorbance #2*	*Mean*
Negative	*0.131*	*0.128*	*0.129*
Standard Deviation	*0.131-0.129*	*0.128-0.129*	
	= 0.002	*= -0.001*	

Mean Standard Deviation = $\sqrt{(0.002)^2 + (-0.001)^2 / 2} = 0.0014$

Therefore Cut-off = Mean + 3*SD
= 0.129 + 3* 0.0014
= 0.129 + 0.0042
= 0.133

Positive Samples	**> Cut Off**
Negative Samples	**<= Cut Off**
Positive Control Value	**> Cut Off**
Negative Control Value	**Absorbance < 0.5**

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Safety Precautions:

- **This kit is For Research Use Only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.

- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
    - Do not smoke, eat or drink while handling kit material
    - Always use protective gloves
    - Never pipette material by mouth
    - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

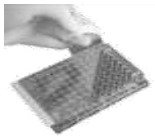
Components (2-8ºC) → 30 mins RT Before Use → 

Thaw at Room Temperature (18-24ºC) → USE NOW

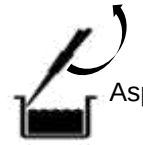
2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) → THAW (22±4ºC) → COOL (2-8ºC) → AVOID → NO CHANGE IN RESULTS

3.  Pipette **100 ul** reconstituted **Positive Control, Negative Control** and **diluted Samples** into the respective wells.

4. Cover plate  and **incubate** for 

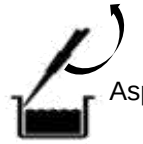
at Room Temperature.

5.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

6.  Pipette **100 ul Anti-Human IgM:HRP Conjugate** into each well.

7. Cover plate  and **incubate** for 

at Room Temperature.

8.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

9.  Pipette **100 ul TMB Substrate** into each well.

10. Cover plate  and **incubate** for 

at Room Temperature.

11.  Pipette **100 ul Stop Solution** into each well.

12. Read absorbance at 450nm with a  microplate reader within 

of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Results
1A 2A	Positive Control Positive Control			
1B 2B	Negative Control Negative Control			
1C 2C	*Sample* *Sample*			
1D 2D	*Sample* *Sample*			
1E 2E	*Sample* *Sample*			
1F 2F	*Sample* *Sample*			
1G 2G	*Sample* *Sample*			
1H 2H	*Sample* *Sample*			

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THANK YOU FOR USING KRISHGEN PRODUCT!

SYMBOLS KEY

MTP	Recombinant SARS-CoV-2 (Covid-19) spike protein Coated Microtiter Plate
PC	Anti-Human SARS-CoV-2 (Covid-19) spike protein Positive Control
HRP CONJ	SARS-CoV-2 (Covid-19) Antibody:HRP Conjugate
CNTRL DIL	Control Diluent
1X ASY DIL	Assay Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalogue Number
	Expiration Date
	Storage Temperature